ISSN 0355-1180

UNIVERSITY OF HELSINKI

Department of Food and Nutrition

EKT Series 1877

THE SENSORY AND PHYSICAL PROPERTIES OF QUINOA LICORICE

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Helsinki 2019

HELSINGIN YLIOPISTO - HELSINGFORS UNIVERSITET - UNIVERSITY OF HELSINKI

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Title
The sensory and physical properties of quinoa licorice

Subject
Food Technology (General Food Technology)

Level	Month and year	Number of pages
M. Sc. Thesis	March 2019	92

Abstract

Licorice is traditionally made from sugar, molasses, water, wheat flour, and licorice extract. In the literature review, the focus was on the properties of quinoa, licorice extrusion, sensory and physical properties of licorice and the basis behind sensory evaluation and instrumental testing in product development. In the experimental part, gluten-free quinoa and rice licorice were made and their properties were studied with multiple sensory and instrumental measurements, and the self-made samples were also compared to commercial samples to see the possible differences between them. The hypothesis was that the changes in the recipes and process parameters would result in significant differences between the products. The sensory evaluations included a generic descriptive analysis with the first batch and two separate consumer tests with the two batches. The instrumental testing conducted of compression and extension tests with Instron and the determination of water activity. The data were analyzed with one- and three-way analyses of variance, as well as principal component analysis for the descriptive analysis.

The manufacturing of the quinoa and rice licorice with the twin-screw extrusion was achieved. The generic descriptive analysis revealed that all the self-made samples were described similarly to each other, while the commercial gluten-free samples differed significantly from these. The quinoa licorice was seen sticky and not homogenous in appearance or texture, while the commercial samples were described as more homogenous and more intense in their taste. The consumer tests showed that both panels preferred the commercial samples over the self-made ones and that the quinoa licorice was preferred over the rice licorice. Commercial samples were seen as soft and chewy, while all the self-made samples from both tests were described as hard and grainy. The hypothesis was overruled since the quinoa licorice samples did not have significantly different properties despite the variation in the process parameters and ingredients. The study revealed that at least with these parameters there are no noticeable, significant differences in the sensory or physical properties of quinoa licorice. The commercial samples, however, remain significantly different from the self-made samples.

Keywords
licorice, quinoa, descriptive analysis, sensory evaluation, extrusion

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Tiedekunta
Maatalous-metsätieteellinen

Laitos
Elintarvike- ja ravitsemustieteiden osasto

Tekijä
Vilma Oksa

Työn nimi
Kvinoalakritsin aistinvaraiset ja fysikaaliset ominaisuudet

Oppiaine
Elintarviketeknologia (yleinen elintarviketeknologia)

Työn laji
Maisterintutkielma

Aika
Maaliskuu 2019

Sivumäärä
92

Tiivistelmä

Lakritsi on perinteisesti valmistettu sokerista, melassista, vedestä, vehnäjauhoista ja lakritsiuutteesta. Tämän työn kirjallisuuskatsauksessa keskityttiin kvinoan ominaisuuksiin, lakritsin ekstruusioon, lakritsin aistittaviin ja fysikaalisiin ominaisuuksiin sekä teoriaan aistinvaraisen arvioinnin ja instrumentaalisten mittausten käytöstä tuotekehitykseen. Työn kokeellisessa osassa gluteenitonta kvinoa- ja riisilakritsia valmistettiin ja niiden ominaisuuksia tutkittiin useilla aistinvaraisen tutkimuksen menetelmällä sekä instrumentaalisilla mittauksilla, itsetehtyjä lakritseja vertailtiin myös kaupallisiin gluteenittomiin lakritseihin. Hypoteesina oli, että erot lakritsien valmistustavoissa tai raaka-aineissa johtaisivat eroihin myös aistittavissa ja fysikaalisissa ominaisuuksissa. Aistinvarainen tutkimus sisälsi yleisen kuvailevan analyysin sekä kaksi erillistä kuluttajatestiä. Fysikaalisia ominaisuuksia tutkittiin instrumentaalisilla mittauksilla, joihin kuuluivat puristus- ja vetokokeet sekä veden aktiivisuuden määrittäminen. Kaikkien tulosten analysointiin käytettiin varianssianalyyseja sekä pääkomponenttianalyysia kuvailevaan analyysiin.

Kvinoa- ja riisilakritsien valmistus onnistui ja tarvittavat testit pystyttiin suorittamaan itsetehdyillä näytteillä. Yleinen kuvaileva analyysi paljasti, että itsetehdyt lakritsit kuvailtiin samankaltaisiksi, mutta ne erosivat merkitsevästi kaupallisista gluteenittomista lakritseista. Kvinoalakritseja kuvailtiin tahmeiksi ja heterogeenisiksi ulkonäöltään ja rakenteeltaan, kun taas kaupalliset lakritsit nähtiin homogeenisempinä ja maultaan voimakkaampina. Kuluttajatutkimuksesta selvisi, että molemmat raadit pitivät kaupallisista näytteistä itsetehtyjä enemmän ja että kvinoalakritsit olivat itsetehtyjä riisilakritseja miellyttävimpiä. Kaupalliset lakritsit arvioitiin pehmeiksi ja sitkeiksi, kun taas kaikki itsetehdytlakritsit arvioitiin koviksi ja rakeisiksi. Hypoteesi tuli kumottua, sillä kvinoalakritseissa ei ollut keskinäisiä merkitseviä eroja, vaikka erot valmistuksessa ja raaka-aineissa olivat huomattavia. Tämän tutkimuksen perusteella voidaan todeta, että testatuilla prosessiparametreillä ei saada aikaan aistittavia tai fysikaalisia eroja kvinoalakritsien välille. Kaupalliset lakritsit kuitenkin erosivat itsetehdyistä näytteistä merkitsevästi.

Avainsanat
lakritsi, kvinoa, kuvaileva analyysi, aistinvarainen arviointi, ekstruusio

Säilytyspaikka
Helsingin yliopiston digitaalinen arkisto Helda

Muita tietoja
EKT-sarja 1877

PREFACE

The experimental research of this study was conducted at the Department of Food and Nutrition in University of Helsinki. The research was supervised by university lecturers Antti Knaapila and Kirsi Jouppila. In the experimental part, support was also given by Jutta Varis and Mikko Kangas.

I would like to thank my primary supervisor Antti Knaapila for the guidance, support and valuable help. I’d also like to thank my other supervisor Kirsi Jouppila and technicians Jutta Varis and Mikko Kangas for all the help during the experimental part, it was very much appreciated.

In addition, I would like to thank my family and friends for all the support that I’ve gotten during this project and over the whole course of my studies. Special thanks to my Viikki grannies and my friends from VSO. I hope you know what you mean to me, without you I couldn’t have done any of this. <3

Helsinki 14.3.2019

Vilma Oksa

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1 Introduction

Licorice root (*Glycyrrhiza glabra*) is a plant, which is widely utilized as an extract. This extract is mainly used in the confectionery industry to produce licorice. Traditional licorice contains sugar, molasses, water, and wheat flour, together with the licorice root extract. Most common additional ingredients are anise aroma and black food colorant (charcoal, E153).

Wheat flour in licorice can be replaced with a range of other flours to get a gluten-free final product. The goal is to get a similar texture than in the traditional licorice by replacing the wheat flour with, for example, rice or oat flour (Clark, 1996). Nowadays, since we are aware that the quality and type of flour used in licorice making has a significant impact on the texture of the licorice, this is a challenge for the industry to overcome (Fayose et al., 2014).

Quinoa (*Chenopodium quinoa Willd*.) is a South American based plant, which is naturally gluten-free and rich in protein (Abugoch, 2009). It has a great amino acid composition and the total protein content (13-15%) is an important nutritional factor. This gluten-free pseudocereal has become an interesting ingredient in the production of gluten-free cereal products such as bread, snacks, and pasta. Gluten-free licorice is highly demanded by the consumers, and quinoa is a great replacement for the traditional wheat on the ever-growing market of gluten-free products (Laurila and Saarinen, 2018). Quinoa licorice has been actively researched in the last few years in University of Helsinki at the Department of Food and Nutrition, especially by Rytkönen (2017) and as pre-tests for this study. These studies reported how quinoa licorice can be produced with twin-screw extrusion and how this type of novel licorice has been perceived by the consumers.

Licorice is traditionally made by cooking in a batch process, but nowadays most industrial scale licorice is made in a continuous process with extrusion. Extrusion is used in the making of breakfast cereals, snack products and confectionery (Jouppila, 2016). In extrusion, the material is conveyed through the screw and simultaneously transformed due to the pressure and shearing in the chamber. Licorice is usually made with a twin-screw extruder because it is better suited for a wider variety of products, especially for oily, sticky and wet extrudates such as licorice (Fellows, 2009). Basing on the literature study Rytkönen (2017) suggested that the most important extrusion parameters for licorice making are mass flow, water content and the rotation speed of the screw.

In the process of creating a new product, sensory studies give crucial information about the consumer response to the product and its properties. Sensory evaluation can be divided into three categories; discrimination tests, descriptive analysis, and affective tests, also known as hedonic tests (Lawless and Heymann, 2010). Discrimination tests address the question of whether there is any difference between samples, by using instructed panels for the evaluation. Descriptive analyses are done by training a panel to evaluate and differentiate specific sensory characteristics from the products. Hedonic tests are done as consumer tests to give a bigger audience a chance to express their views on the pleasantness of the products (Moskowitz, 2011). All evaluation techniques are widely used in product development. Mechanical tests are equally important while determining the properties of a new food item in product development (Shama and Sherman, 1973). With confectionery and especially licorice, extension and compression tests help to compare samples with each other and create a better understanding of the physical properties of the matrix (Olkku and Rha, 1975). The physicochemical properties of the licorice are also studied, by determining the water activity since it tells a lot about the reactivity and stability of the product (Kallio, 2006).

There has not been much research on the sensory properties of licorice and therefore this study was seen necessary. The literature review of this study aimed to clarify the properties of the ingredients in licorice, especially quinoa and the process of licorice extrusion. It also focused on some of the properties of the actual licorice and explained the use of sensory evaluations and mechanical testing in product development. In the experimental research, gluten-free quinoa and rice licorice were made with a twin-screw extruder and their properties were studied with various sensory evaluations and other analyses. A generic descriptive analysis was used to conduct a full sensory profile of the quinoa licorice and two consumer studies were arranged in order to study the pleasantness of all samples. Water activity of the licorice was determined, and mechanical properties of the quinoa licorice were studied with compression and extension tests.

2 Literature review

2.1 Licorice ingredients

2.1.1 Flour

Traditional licorice is made with wheat flour, but nowadays a bigger variety of different flours have emerged into the markets due to product development and consumers' demand for different raw materials. Especially gluten-free options replacing the traditional wheat have been under research under the last decades, and it has resulted in a selection of different licorice such as oat and rice licorice for example.

The typical flour content in licorice varies between 20-40% (Groves, 1998). Most flours contain around 60-70% starch, and starch is a polysaccharide consisting of thousands of glucose units that act as carbohydrate storage in green plants (Fennema et al., 2008). Starch consists mainly from amylose and amylopectin and it exists in cereals and other plants filled with carbohydrates, such as potato and cassava. Starch contains usually around 20-30% of amylose and 70-80% of amylopectin (Atkins, 1987). Starch is present as starch granules in the plants and the size and shape of the granules vary among plants (Fennema et al., 2008). The granules are not soluble in cold water, but they do swell up to 10-15% bigger in water.

The flours used in licorice making affect the final structure of the licorice since starch forms a gel in the process and the degree of gelatinization has a huge effect on the final structure (Edwards, 2000). The elastic structure and glossiness of the final product is the better, the higher the degree of starch gelatinization is (Anzmann, 2016). Also, the quality and type of flour used in licorice making have a significant impact on the texture of the licorice (Fayose et al., 2014). The goal is to have a similar texture in the licorice regardless of the type of flour used, and even if gluten in the flour can impact the texture, the main part of controlling the licorice texture relates to starch gelatinization (Hartel et al., 2017). The protein content of the flour can affect the final product, as well as the falling number. According to Aalto-Kaarlehto (1993), the flour in licorice manufacturing should have a high falling number in order to result in low content of amylose, which is preferable in licorice making. The falling number can be tested from the flour. If the amylose content is high, the testing time is short, but with licorice making the amount of amylose needs to be low and therefore the testing time longer (Edwards, 2000). The minimum falling number for licorice making is 200, but higher values are also appreciated.

Quinoa

Quinoa (*Chenopodium quinoa* Willd.) is a pseudocereal that originates from the Andean region (Figure 1). It is cultivated nowadays around the world, including Finland, even though most of it is still grown in its origin countries in South America (Abugoch, 2009). It has legume-like characteristics as well as cereal-like properties, however, quinoa is neither a cereal nor a legume. It is a starchy dicotyledonous seed and therefore referred to as a pseudocereal. Quinoa seeds can range from yellow to pink and black in color, while they always are flat and oval-shaped. According to Fleming and Galwey (1995), the seeds are quite small, varying from 1 to 3 mm in size.

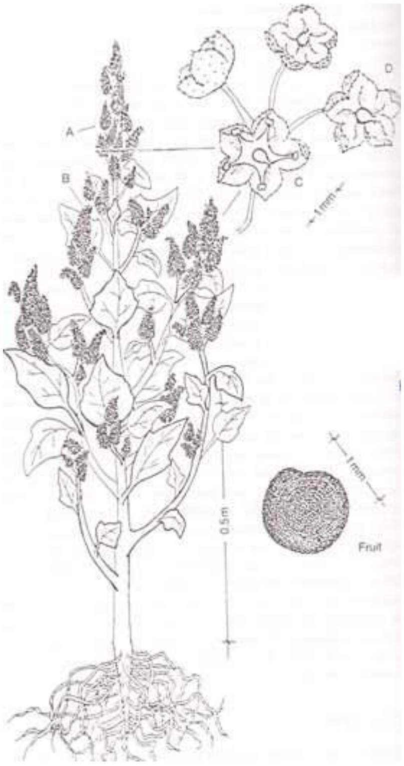


Figure 1. Quinoa plant (Fleming and Galwey, 1995)

Quinoa is also rich in protein with an overall protein content of 13-15%, which gives it a high nutritional value. The total protein content of quinoa seeds is higher than in other cereals such as barley, rice or corn and it is comparing well to wheat (Abugoch, 2009). The compositions of quinoa, wheat and rice flours can be seen in Table 1. Quinoa proteins are also high in lysine, which is an essential amino acid and it is usually absent in most cereal grains. The total amino acid balance of quinoa proteins is great compared to other cereals and legumes, since they contain all of the 20 different amino acids, with a high percentage of lysine and methionine (Abugoch, 2009). Quinoa's protein content and distribution of essential amino acids resemble the biological value of milk proteins (caseins) and is valued better than that of other cereals (Filho et al., 2017). The amino acid composition is close to the ideal protein equilibrium stated by FAO, and the high amount of lysine makes it a protein complete food. The nutritional value of food is largely determined by protein quality (Comai et al., 2005). Protein quality depends on amino acid content, digestibility, and influence of antinutritional factors among other things. Ruales et al. (1993) found that the protein digestibility of raw and washed quinoa was 83% when casein has 91%.

Gluten is a combination of storage proteins in starch endosperm of some cereals (Wieser, 2007). Gluten includes prolamins (gliadins, hordeins, secalins, avenins) and glutelins. Typically, just the prolamins are referred to as gluten and glutelins as glutenin, especially when referring to wheat. Quinoa is naturally gluten-free as it lacks gliadin proteins and the protein fractions corresponding to gliadin (Filho et al., 2017). This makes it a great option for producing food items suitable for people with celiac disease or wheat-allergy as well as others who want to reduce the amount of gluten in their diet.

Table 1. The composition of quinoa, wheat and rice flour (adapted from Ogungbenle, 2003 (quinoa); Bock and Flores, 2011 (rice); Arnarson, 2015 (wheat))

Component	Content (g/100g)		
	Quinoa	Wheat	Rice
Water	11.2	10.7	10.8
Carbohydrates	58.3	72.0	79.0
Protein	13.5	13.0	6.0
Fat	6.3	1.0	1.4
Fiber	9.5	2.2	2.2
Ash	1.2	1.1	0.6

In addition to great protein content, quinoa also contains a variety of vitamins, minerals and other compounds such as polyphenols, phytosterols and flavonoids (Abugoch, 2009). Quinoa has been shown to have a higher antioxidant activity than some cereals, such as rice and buckwheat (Gorinstein et al., 2008). Quinoa, being a pseudocereal, has been considered in the industry also as an oil crop. It has notable contents of vitamin E and a great proportion of omega-6 fatty acids, making the oil fraction highly nutritious and high-quality (Abugoch, 2009). Quinoa also contains 58-64% starch and it has some functional and physicochemical properties that allow quite diverse uses for it. These properties include solubility, water-holding capacity, gelation, emulsifying, foaming, viscosity and freeze stability (Abugoch, 2009). Quinoa starch is high in amylopectin (<77%) and low in amylose (11%), with a total gelatinization temperature of 57-64 °C (Fleming and Galwey, 1995). Due to the low amount of amylose in quinoa starch, it does not retrograde easily. According to Taylor and Parker (2002), the quinoa starch granule is only 0.4-2 μm in diameter and polygonal in shape. The small size of the starch granule makes quinoa dough unstable and it does not have good baking qualities, which is also a result of the lack of gluten. The poor baking quality has resulted in seeking other uses for quinoa, such as snacks and breakfast cereals (Abugoch, 2009).

Quinoa plant contains saponins, as a protective feature on the outer layers of the seeds (Abugoch, 2009). Saponins are steroid or triterpenoid glycosides and they are usually removed by brushing and rinsing the seeds (Francis et al., 2002). The amount of saponins in quinoa has been difficult to determine, but the general values found in the literature vary between 0.1-6% (Cheok et al., 2014). Saponins are toxic in large amounts and they have a very bitter taste, which is why they need to be removed before eating or processing quinoa seeds any further. In addition, the elimination process reduces the amount of phytic acid in quinoa approximately by 30% (Abugoch, 2009). The amount of phytic acid in processed quinoa seeds is lower than in whole grains like wheat, lentils and fava bean (Filho et al., 2017).

Rice

Rice *(Oryza sativa L.)* is one of the most widely grown cereal crops for food (Hasjim et al., 2013). Rice is a monocot plant, which needs ample water for growing and hard workers for the labor-intensive cultivation (Figure 2). Rice grains are mainly consumed as a whole grain, usually polished, but broken grains are typically milled and used as flour (Singh et al., 2014). Due to the consumption of the intact grains, the physical properties such as size, shape, and uniformity are truly important with rice (Elbashir, 2001). Rice plant is a grass plant and it is the most important grain to human nutrition worldwide. The leaves are long and slender, and the grains are normally 5-12 mm long and 2-3 mm thick.

Figure 2. Rice plant (Stock Illustrations, 2018)

Rice contains mostly starch (over 80% of the dry weight) and therefore starch is the factor that determines the quality of rice products (Hasjim et al., 2013). Rice starch contains typically 19% amylose and the rest is amylopectin at 81% (Huang and Rooney, 2001). Compared to wheat and quinoa (Table 1), rice contains more starch, but fewer proteins (ca. 6%) and a third of the amount of fat or fiber when compared to quinoa (Bock and Flores, 2011). The cooking characteristics of rice are influenced by the starch as well as the non-starch constituents (Juliano et al., 1964). One of the most important factors for the cooking quality of rice is the gelatinization temperature (Rao et al., 2004). Rice starch gelatinizes between temperatures 68-78 °C (Huang and Rooney, 2001). For some varieties, soaking improves the texture of the cooked rice by increasing expansion of the grains (Juliano et al., 1964).

Cham and Suwannaporn (2010) stated that native rice flour has a low elastic gel-forming ability and a poor resistance to shear force. The power of viscosity and thickening are usually lost during cooking from native starches such as rice. The quality of rice gel depends on the variety of the grain, the amount of amylose in the grain and the aging period (Meullenet et al., 1998). The aging of rice means the changes in physical and chemical properties of rice grains that occur during storage (Zhou et al., 2003). The aging happens before harvest and it can affect especially the texture and the flavor of the rice.

Physicochemical properties of rice such as hydration, swelling, solubility and viscosity change during storage. Stack burning can occur when wet grains are piled together without proper aeration (Juliano, 1985). This stack burning results in yellow rice, due to the microbial infestation, and this reduces the amount of lysine in the rice up to 10%. There are some benefits from long-term rice storage as well. During the postharvest ripening, the endosperm becomes harder, which results in better yield in milling and better-quality rice after cooking (Villareal et al., 1976).

2.1.2 Sugars

Typical licorice contains different sugars, such as granulated or liquid sugar, invert sugar and molasses. In licorice confectioneries, the normal amount of those sugars is around 50-60% (Minifie, 1997). According to Smullen (1991) reducing sugars should be used in licorice making, since they create the wanted shine for the product and stabilize the water content in the licorice dough. Reducing sugars also have an effect on the taste of the product. Reducing sugars are the sugars that interact with amino acids, resulting in Maillard reaction and thus creating browning and aroma formation (Tester and Karkalas, 2003).

Molasses is the residue fraction of crystallized sucrose, which includes plenty of reducing sugars and therefore, it is used widely in licorice making, contributing to the signature taste (Edwards, 2009). Molasses increases the shelf-life of licorice by lowering the water activity (Pennington and Baker, 1990). Molasses also acts as a stabilizer in licorice, diminishing the interactions between ingredients and preventing the ingredient separation. The sucrose is partly inverted into glucose and fructose in molasses (McNeill, 2004). Molasses are also dark in color, strong in flavor and thick in texture (Edwards, 2009). The content of solids varies between 80-81% in molasses and from that 20-28% are invert sugars. When making licorice with a twin-screw extruder and co-rotating screws, granulated sugar is not advised due to the poor solubility in that case (Meuser and Wiedmann, 1989).

2.1.3 Licorice root

Licorice root is used as a natural sweetener and flavoring agent around the world (Kitagawa, 2002). The root is a part of a leguminous plant grown mostly in Mediterranean climate and Middle-East (Casulli and Ippolito, 1995). The root is grayish brown and around 15-20 cm long (Figure 3) and the plant's name means “sweet root” in Greek (Edwards, 2000).

Figure 3. Licorice root plant (Størmer et al. 1993)

Licorice root plant contains various sugars, starches, flavonoids, sterols, amino acids, gums and essential oils (Snow, 1996). It has an active ingredient called glycyrrhizin, a triterpenoid glycoside, which makes up to 14% of the total soluble content of the root (Baran and Fenerciogaelu, 1991). This substance gives the licorice root its characteristic sweet taste, since glycyrrhizin is 50 times sweeter than cane sugar, and it has a slight licorice flavor and tanned color. According to Belitz (2009) confections have to have at least 5% of licorice root extract in order to be identified as licorice confectionary.

There are a few advantages for glycyrrhizin use: the sweetness is sensed slowly in the mouth, but the sensation lingers for a long time (Attokaran, 2011). The substance also maintains the sweetness even after heating, which is especially important in confectionery manufacturing. Glycyrrhizin is also used as a sweetening agent, flavor enhancer, flavor modifier or foaming agent (Blomberg and Hallikainen, 1993). However, due to the strong and distinctive flavor of glycyrrhizin, its use in foods is limited, and licorice root is mainly used only in confectionery. The substance also gives the products an undesirable brown color and the sweetness is lost in acidic solutions such as beverages (Isbrucker and Burdock, 2006). Licorice extract is processed from the plant root by boiling it in water (Clark, 1996). The extraction is filtered so that the insoluble particles can be taken out and then it is concentrated by evaporating the water. According to Attokaran (2011), the final, paste-like licorice extract contains 80-85% dry substance. From that 30-40% is starch and gums, 16% sugar and 12-20% glycyrrhizin. The exact composition is hard to predict since it varies widely based on the cultivation conditions, environment, and species (Duke, 1985).

A disadvantage of the glycyrrhizin is, that it inhibits the enzyme responsible for cortisol inactivation in the body (Isbrucker and Burdock, 2006). Therefore, continuous high-level exposure to these compounds can produce hyper-mineralocorticoid-like effects in humans, which usually occurs as high blood pressure. However, the effects are reversible if the consumption of licorice or glycyrrhizin is withdrawn. An acceptable daily intake of 0.015-0.229 mg glycyrrhizin/kg body weight/day is proposed (Isbrucker and Burdock, 2006). The estimated average intake of glycyrrhizin in the USA is less than 2 mg/day, but acute exposures can occur as the result of eating large amounts of licorice candy. The European Union has stated already in 1991, that the proposed safe daily intake for glycyrrhizin is 100 mg/day, which concludes around 60-70 g of licorice per day (Murphy et al., 2009).

2.1.4 Water

In the manufacturing process of licorice, the structure can be controlled with water content (Clark, 1996). The total water content of the final product affects the texture so that, with the used amount of water the dryness, hardness and stickiness can be affected. Chinachoti et al. (1990) stated that starch gelatinizes when there are 0.4 grams of water for every gram of starch and that the perfect gelatinization happens when there are 1.5 grams of water for every gram of starch. At the beginning of licorice cooking, the water content of the licorice dough is typically around 40%, and there is 3.6 grams of water for every gram of starch (Aalto-Kaarlehto, 1993). During the cooking process, however, more than half of all the water evaporates. Smullen (1991) suggested that even less water is needed while making short licorice pieces. In those cases, 30-32% of water content should be sufficient, while 47% of water content would be advisable for longer licorice manufacturing.

Water content effects also the shine of the licorice. Kallio (2006) and Rytkönen (2017) stated that, when the water content of the dough increases, the shine of the licorice increases as well. The usual water content in licorice is 16-20%. Sometimes preservatives are used in licorice making to inhibit mold growth, which can occur if the water content is too high in licorice. According to Bussiere and Serpelloni (1985), the water activity of licorice should be around 0.5-0.75 in order to inhibit microbial growth. Water activity tells the amount of free water, which is usable for microbiological or chemical reactions.

2.1.5 Additives

In licorice manufacturing, a variety of food additives can be used (Smullen, 1991). The usual additives in Finnish licorice are black food colorant (E153) and anise oil. The black colorant is all natural, activated charcoal, and it is used to disguise the unpleasant brown color of the licorice. Another common additive in licorice manufacturing is anise oil, and especially the traditional black licorice is typically flavored with anise oil. Anise is an aromatic plant and anise oil is extracted from the dried anise seeds typically with a hydrodistillation process (Özcan and Chalchat, 2006). Other additives can also be used when making licorice. Vegetable gums can be used as thickening agents and gelatin has also been used as a binding agent for water and for preventing cracking of the surface (Subramaniam, 2011). Sometimes various emulsifying agents, acidity regulators, and preservatives are used, and different waxes are used for polishing of the licorice to additional shine (Lähteenmäki et al., 1996). Salt is normally used in tiny amounts (0.1%) in the licorice dough since it enhances the final flavor (Jackson, 1990). Sweeteners can be used in licorice to enhance sweetness or substitute the sugars.

2.2 Licorice manufacturing

Licorice is made by mixing the ingredients, cooking the dough and then shaping the licorice to the wanted shape (Groves, 1998). The final product needs to be dried and is usually finished off with polishing or coating. Flours make 20-40% of the licorice dough and sugar an additional 30-40% (Olkku and Rha, 1975). Water content is typically higher in the beginning, but after the cooking process the water content of the licorice is 30% and after drying it settles around 15-25%. Typical black licorice is dark brown or black in color and it is flavored with anise oil and licorice extract.

Licorice can be made as a batch process by cooking in a pot or with continuous processes with heat exchanger or extrusion (Groves, 1998). In the batch cooking process, the control over starch gelatinization is better and therefore the wanted texture is more achievable, but with the continuous processes, the advantages are shorter processing times and smaller equipment (Smullen, 1991). According to Groves (1998), the main advantages for extrusion are the continuous processing, energy-efficiency and better impact on the texture and flavor of the product.

2.2.1 Extrusion in general

Extrusion is a flow-through cooker that combines several unit operations. Berk (2009) showed that the extrusion cooking is a thermomechanical process, where, in one process heat transmission, mass transmission, pressure variation and shearing result into boiling, sterilizing, drying, melting, mixing, kneading and cooling. The material is conveyed with the screws and transformed, due to the pressure and shear in the chamber. Riaz (2006) divided the extruder into three processing parts: feeding, kneading, and cooking. Feeding typically includes only the solid ingredients, since the liquid is pumped in only at the beginning of the kneading section. The density of the product grows when it squeezes through the kneading section with the screws. The cooking section’s goal is to condense and push the product towards the nozzle. The high pressure, shearing forces from the screws and heat transform the material to a semi-solid, plastic mass. According to Fellows (2009), the most important parameters in extrusion are temperature, pressure, shearing forces, screw rotation speed and the diameter of the nozzle.

Extruders can be divided into single- and twin-screw extruders, even if the production principles are the same. A twin-screw extruder can work a bigger variety of raw materials since it is better suited for oily, sticky and wet materials because they are carried through better than in a single-screw extruder (Fellows, 2009). Other advantages for a twin-screw extruder over the single-screw one is shorter processing time, ability to higher shear forces, better temperature control and the self-cleaning ability of the screws. According to Ainsworth and Ibanoglu (2006), screws that are overlapping and moving in opposite directions are suited for materials with low viscosity, because they need a low screw rotation speed and a long processing time in the extruder. Such materials are confectionery products like chewing gum, jellies and licorice. The screws rotating into the same direction are used in processes that require a more intense heat transfer. However, extrusion with screws rotating into the same direction is possible, if granulated sugar is changed into liquid sugar (Meuser and Wiedmann, 1989). With screws operating into the same direction, the processing time would have to be a lot longer when using granulated sugar, in order to get it dissolved in the process, but with liquid sugar, the processing time can be kept shorter like with the opposite direction screws.

2.2.2 Licorice extrusion

It is possible to make licorice with extrusion. The use of extrusion has found its applications not only in the pre-processing of the components but also in the manufacturing of finished confectionery products (Moscicki, 2011). The solid ingredients (flour, licorice extract) and the liquid ingredients (water, molasses, and liquid sugars) are mixed separately together before the extrusion (Meuser and Wiedmann, 1989). They can be fed into the process together at the feeding screw, or separately so that the liquid ingredients are fed further on with a pump. The flow chart of licorice extrusion is seen in Figure 4.

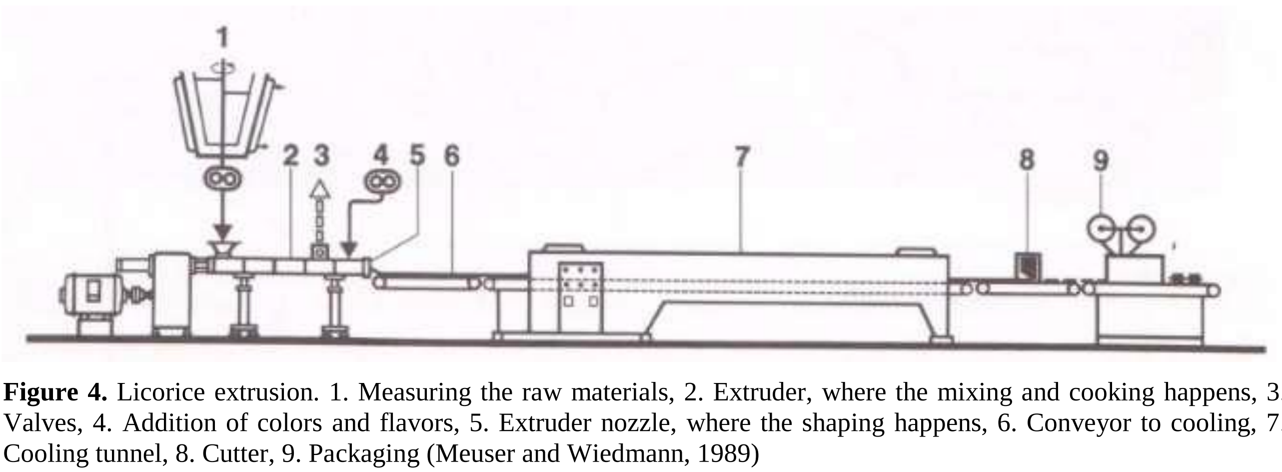


Figure 4. Licorice extrusion. 1. Measuring the raw materials, 2. Extruder, where the mixing and cooking happens, 3. Valves, 4. Addition of colors and flavors, 5. Extruder nozzle, where the shaping happens, 6. Conveyor to cooling, 7. Cooling tunnel, 8. Cutter, 9. Packaging (Meuser and Wiedmann, 1989)

In the extruder, the dough gets pushed by the screws forward into the chamber and the temperature is controlled by heating the sheath (Riaz, 2006). When the starch starts to gelatinize, the pressure and viscosity start slowly increasing, which lead to rapid increases of temperature and shearing forces. The pressure increases up to 14 bar and the temperature to 150 °C. This is necessary for the sugars to dissolve completely and the starch to gelatinize and plasticize (Meuser and Wiedmann, 1989). The hot licorice mass is cooled down a little in the middle of the process, with the help of a valve. Opening the valve creates negative pressure by letting some water evaporate, and this cools down the product enough so that heat sensitive ingredients (colorants, aromas) can be added. The temperature drops to 90 °C during cooling, but it is increased again a little before pushing out the licorice from the nozzle.

Groves (1998) stated, how cooking in the extruder results in better gelatinization of starch than with heat exchangers since the shearing forces are bigger. Also, with an extruder the process stays better under control, the exchange of products is faster, and the cleaning of equipment is easier, compared to batch processes and heat exchangers (Ainsworth and Ibanoglu, 2006). Hoseney (1994) stated that gelatinization forms more quickly and perfectly, the more water there is in the gelatinization process. It is known, that gelatinization is successful when there is at least 30% water and the temperature is higher than 65-70 °C. The extrusion of starchy foods results in gelatinization, partial or complete destruction of the crystalline structure and molecular fragmentation of starch polymers, as well as protein denaturation, and formation of complexes between starch and lipids as well as protein and lipids (Hagenimana et al., 2006).

The size and shape of the starch granules also affect the extrusion process (Guy, 2001). Smaller granules heat up faster to the critical solubility point since the heat goes through them faster than through the bigger granules. A small amount of swollen starch granules is desired for licorice because that creates the wanted smooth, firm and chewy texture and shiny appearance (Hartel et al., 2017). When starch granules are not sufficiently gelatinized (swollen, but remaining essentially intact), the resulting licorice is firm and dry. Then again, if the starch granules are over-gelatinized (completely fractured), the resulting licorice is limp and elastic, with a tight, chewy consistency.

2.2.3 Effects on licorice properties

The sensory properties of licorice have not been studied a lot. Licorice contains mainly sugars, starch, and protein, all of which affect the overall properties and the acceptance of the product (Clark, 1996). Licorice can be affected by the variety of flour and the amount of flour used, in addition, the amounts of used sugar and water can affect the final product. Olkku and Rha (1975) also stated that licorice is viscoelastic and that the final product should be chewy. Stickiness is one formidable and typical property of licorice, and according to Rokey (2011), the stickiness of the end-product can be controlled by altering the water content of the dough and the amylose/amylopectin ratio, or by lowering the shearing forces in the extrusion. Thomas and Atwell (1999) also showed that native starches cannot handle high temperatures and shearing forces that occur during extrusion, and that is why the breaking of starch granules leads to a sticky product. Process parameters in licorice extrusion such as screw rotation speed, temperature, and mass flow all have an influence on the properties of the final product. They mostly affect hardness, diameter, shine and flavor (Müller, 2012).

Starch gelatinization has a huge effect on the sensory properties of licorice. The texture of the final product can be altered by varying the degree of gelatinization of the starch (Edwards, 2000). Licorice products vary for example in their elasticity due to the differences in the starch gelatinization. According to Jackson (1990), the texture and shine are better in licorice, where gelatinization has been successful, compared to licorice where gelatinization has not been sufficient. Edwards (2000) also stated that the shine of licorice reflects the degree of the gelatinization. The proteins, lipids, added sugars, and salts all affect the gelatinization, since every time something like sugar or salt is added to water it affects the thermodynamic properties of water. This type of behavior can affect water's capability to interact with other components in the system according to Spies and Hoseney (1982). If the reactivity of the water grows low, the chemical and physical reactions need more energy. In the process of making licorice, the addition of sugars affects the gelatinization the most.

Water content affects the properties of licorice in a significant way. Some control over the licorice texture is achievable by altering the water content of the final product (Edwards, 2000). According to Müller (2012), the hardness of the licorice increases when the water content is increased. This effect is dependent on the increase of the feeding rate as well. When the screw rotation speed is increased, while the water content decreases, the viscosity of the licorice gets bigger and the pressure increases. This leads to a more solid and hard final product. Kallio (2006) stated that the higher the water content in the licorice, and the higher the temperature during the manufacturing, the darker the color and the better the shine in the final product. Also, if the water content is high and the feeding rate is low at the same time, the diameter of the licorice increases.

2.3 Sensory evaluation

Sensory science is a discipline, where scientific methods are used for evoking, measuring, analyzing and interpreting sight, smell, touch, taste, and hearing responses from various products (Stone et al., 1974). Meiselman (1993) established that sensory evaluation is a science of measurement, where it is concerned with precision, accuracy, sensitivity, and avoiding false positive results. There are three categories of sensory testing, and they all use different types of panels and aim for separate goals: discrimination tests, descriptive analyses, and hedonic tests (Lawless and Heymann, 2010). These three kinds of sensory evaluation methods focus on the existence of overall differences among products, the specification of sensory attributes and their intensities, and measuring consumer likes and dislike.

The first type of sensory evaluation tries to answer whether any observable difference exists between two samples (Lawless and Heymann, 2010). These simplest tests include discrimination tests and simple difference testing procedures such as triangle test, duo-trio-test and paired comparisons (Peryam and Swartz, 1950). These difference evaluations usually include a panel of 25-40 participants (Moskowitz, 2012). A panel of this size is generally adequate for detecting clear sensory differences and the data analysis of these tests are quite simple to conduct.

The second type of sensory methods is those, where the intensities of the sensory characteristics of a product are quantified (Lawless and Heymann, 2010). These sensory evaluations are known as descriptive analyses and they have proven to be the most extensive, comprehensive and informative sensory assessments. There are plenty of different descriptive analyses, that are used for different purposes, such as the Flavor Profile® (Caul, 1975), the Texture Profile (Brandt et al., 1963; Szczesniak et al., 1975) and the Quantitative Descriptive Analysis® (Stone and Sidel, 2004). The complexity of these studies requires a panel of 10-12 participants, who are well-trained, given practice and know the meaning of all the terms and descriptions they need to use for the evaluations (Moskowitz, 2012).

The third type of sensory evaluations is known as hedonic or affective methods since they try to quantify the degree of liking or disliking of a product (Lawless and Heymann, 2010). These tests are conducted as consumer tests and aim typically for a panel of 75-150 consumers who are typically regular users or potential users of the product. A large panel is needed due to the high variability of individual preferences and by using a bigger sized panel these variations can be compensated using statistical analysis (Moskowitz, 2012). A larger panel can also give information about the reasons for liking or disliking of a product with all the demographic information. It is also a known fact, that only human sensory data provides the best response for consumer perception and reaction to foods in real life.

Sensory evaluations can also be divided into two groups based on panel-types, analytical laboratory measurements with trained panels and hedonic tests as consumer evaluations (Tuorila and Appelbye, 2008). Both of these groups of sensory evaluations (analytical and hedonic) are used in product development. With the analytical evaluations (descriptive or discriminating) the manufacturer gets to know what sensory properties the product includes, and with the help of a hedonic test it can be seen which of those properties are liked or disliked amongst the consumers. In the following paragraphs, descriptive and hedonic sensory analyses will be presented in more detail.

2.3.1 Descriptive analyses

Descriptive analyses are analytical sensory tests that focus on the sensed properties of the product and quantify the intensities of the sensory characteristics (Lawless and Heymann, 2010). When these tests are used correctly, they can produce full sensory profiles from the studied products. Descriptive analyses are mainly used in product development and quality control in the real world and they can be quantitative or qualitative (Tuorila and Appelbye, 2008). Most of the times both styles (qualitative and quantitative) are used together, firstly describing the product characteristics qualitatively and then evaluated using quantitative methods (Moskowitz, 2012). Generic descriptive analysis is probably the most versatile method since it is a modification of different descriptive analyses (Tuorila and Appelbye, 2008). The panel in descriptive analyses consists out of 10-12 people but sometimes, if the described differences are known to be small, a larger panel might be needed. A panel of fewer than 10 people can lead to biased results since the sampling is limited (Lawless and Heymann, 2010).

In the generic descriptive analysis, the panel is trained, preferably in multiple sessions, to be as objective and analytical as possible. The training begins with the panelists individually evaluating the samples and then the panel leader combining the descriptive attributes into one vocabulary (Lawless and Heymann, 2010). The panelists must identify, name and describe the properties. The goal for the vocabulary is to be clear for all panelists and to describe the samples so that the words separate the samples from each other as well as possible (Moskowitz, 2012). There is no reason to use words that do not separate the samples or can be interpreted in multiple ways since this increases the possibility of errors and false positives.

Sometimes reference samples or verbal explanations need to be used for better clarification. No words suggesting preference or liking can be used since the panel needs to perform as objective as it can (Tuorila and Appelbye, 2008). Most of the times, line scales are used with numeric or verbal anchors. With these kinds of evaluations, unipolar scales with verbally anchored ends are the most common (Moskowitz, 2012). They keep the descriptive attributes clear and separate, and the evaluation is easier with verbal anchors. With descriptive analyses, at least two evaluation sessions are necessary, preferably even three for better repetition and reliability with the results (Lawless and Heymann, 2010). A generic descriptive analysis gives information in quantitative and qualitative ways, and the data analysis usually includes average results in spider-charts, variance analysis, and principal component analysis.

2.3.2 Consumer tests

There are two main approaches to consumer sensory testing, the preference tests, and the acceptance tests, both of which are used for food and consumer product evaluations (Jellinek, 1964). In preference test the panelist has a choice, one product is to be chosen over one or more products. In an acceptance test, the panelist rates their liking of a product on a scale. Both of these types of tests are called hedonic or affective testing and they refer to pleasure and the pleasantness of the samples.

A successful consumer test needs to have a good panel, with the right participants (Lawless and Heymann, 2010). The panelists need to represent the wanted generalized population and they should be frequent users of the evaluated products. The wrong type of panel can make the whole consumer test useless for further product development. The main goal with a consumer test is to see if consumers accept a product or which product they prefer over the others (Moskowitz, 2012). Typically, a consumer test is conducted when a new product enters the market, the recipe or manufacturing of an existing product is changed or there is a need for comparison between competitors.

Consumer tests can be arranged with a local standing panel with people living close to the factory, a central location test in a mall or a home use test (Lawless and Heymann, 2010). Internal employee panel is convenient for the panel and the sensory professional, cost-efficient and secure, but it might not represent the general public and the employees might have bias conceptions about the products. Local standing panels are panels collected from the local people (Moskowitz, 2012). This means the panel is reasonably secure and distribution is easy. However, the local people are not a random sample, and this might cause bias. The panelists might also discuss the products amongst themselves and this could affect the results.

Central location tests might be the most popular ways of conducting a consumer study. This type of test gives a representative sample of the public since it is open for everyone and usually arranged in an open location with easy access (Moskowitz, 2012). The disadvantages include the need for separate testing agencies and location as well as the possible costs and slow rate of data collecting. Another category of consumer test is home use tests (Lawless and Heymann, 2010). The consumers take the products home and try to use them as they would after buying them from the supermarket. This gives also a good, representative sample and realistic testing. This way the whole family input is ensured and the consumers get to also test the user directions accompanied with the product. This type of testing includes, however, a security risk with the products, lacks product control and it is by far the slowest and costliest way of testing the products (Moskowitz, 2012). The choice of which test to use in a situation is usually a compromise between time and expense, as well as the need for the best information about the product.

Consumer tests include the actual evaluation questions, but they also typically have a section that determines some background information about the panel (Moskowitz, 2012). These questionnaires most likely have general questions about the panelists’ age, gender, marital and social status or something else that might concern the researchers. The demography of the panel might even have an effect on the results and the way the panel sees the product (Lawless and Heymann, 2010). It is important, that the questionnaire for background information is done politely and kept short enough. Consumer test questionnaires are also kept rather simple in their appearance and the questions in the actual evaluation need to be understandable for a panel that has no previous sensory evaluation experience (Moskowitz, 2012). This means, that scaling a consumer test is extremely important. The most common scale used for consumer testing is the 9-point hedonic scale, since it has a centered, neutral category, and it has scale point labels with adverbs that represent their hedonic counterparts. In other words, it is a ruler-like scale, whose equal intervals are used for statistical analysis.

2.4. Instrumental testing of physical properties

Many sensory properties have counterparts in the physical or chemical properties of the product (Lawless and Heymann, 2010). The physical force that is required to pierce through the sample is related to the detected hardness. The observed thickness, on the other hand, is partly related to the physical viscosity. The texture, or structure as called while talking about the physical state, is sensed with multiple senses and it is not that simple to evaluate as taste and odor (Tuorila and Appelbye, 2008). That is a big reason why a lot of sensory science is simultaneously studied with mechanical testing. This way the data from sensory evaluations can find an equivalent in a physical form.

The rheological properties of a product can relate to deformation, disintegration or vibration from outside sources, while instrumental texture measuring focuses on chewing and the changes it creates (Tuorila and Appelbye, 2008). Typical attributes described with mechanical testing is hardness, fracturability, cohesiveness, springiness, chewiness, gumminess and resilience.

Hardness is described by the biggest force that focuses on the sample during the first bite. The moment when the sample breaks, during the first bite, is described as fracturability. Cohesiveness means the ability of the sample to oppose deformation during the second bite while compared to the first bite. The time that the sample takes to gain its structure during the second bite, compared to the original height, is called springiness. Chewiness is calculated as the gumminess multiplied with the springiness, while gumminess, on the other hand, is the multiplication of hardness and cohesiveness. Lastly, resilience describes how well the sample tries to gain back its original shape.

Instrumental measuring of structure can be used to complement the sensory measurements of texture. According to Szczesniak (2002), the texture is the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch, and kinesthetics. Food texture can be extremely important to the consumer, and therefore, according to Lawless and Heymann (2010), unlike color and flavor, the texture is frequently used by the consumers not as an indicator of food safety, but as an indicator of food quality.

The strength of materials is a subject which deals with the behavior of solid objects when they are subjected to stresses and strains (Howatson et al., 1972). In these mechanics of materials, the strength of a material is its ability to withstand an applied load without failure or deformation. The stresses that act on the material cause deformations in various manners, including breaking them completely, are called strain when they are placed on a unit basis. The applied loads that are forced on to the materials can be axial (tensile or compressive) or rotational (strength shear) (Groover, 2010). Material strength is described as the point in the engineering, at when passed the material experiences deformations that will not be completely reversed anymore upon removing the loading.

A stress-strain curve can be plotted by the measuring instrument and would look similar to the following in Figure 5.

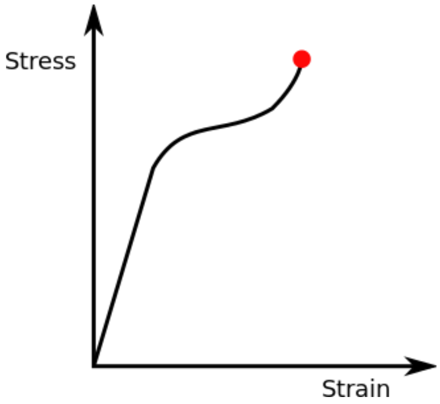


Figure 5. Stress-strain curve (Howatson et al., 1972)

Both compressive and tensile strengths are defined as uniaxial stress σ, which is measured as force F (load applied, [N]) per unit area A [m^2] (Equation 1).

$$\sigma = \frac{F}{A} \tag{1}$$

Compressive strength is the capacity of a material or structure to withstand loads tending to reduce the size (Howatson et al., 1972). When a material is pushed together and shortens under a uniaxial load, it is said to be in compression. Typically, the specimen is then shortened and spread laterally until the material fails completely. Tensile strength, on the other hand, is the capacity of a material or structure to withstand loads tending to stretch and pull the object. When a material is loaded in such a way that it extends under a uniaxial load it is said to be in tension. Typically, the sample is pulled on a constant strain rate until it breaks. Compressive strength resists compression and tensile strength resists tension, and these strengths can be tested with a universal testing machine, like Instron (Groover, 2010).

3 Experimental research

3.1 Aims

The sensory and physical properties of licorice have not been studied widely, and the research concerning gluten-free licorice is practically non-existent. This study concentrates mainly on conducting different sensory analyses in order to create a sensory profile of gluten-free quinoa and rice licorice and to see how self-made samples fair against commercial gluten-free licorice. Extrusion parameters have a big effect on licorice properties. Starch gelatinization should be optimized and the water content of the licorice was chosen based on previous research. These two process conditions have the biggest effect on the properties of the final product. Since taste and texture are important sensory properties in confectionery, this study focused mostly on those characteristics and their effect on the consumer opinions of the samples. In addition, instrumental measurements were used to get more insight into the physical properties of candy licorice.

The goal of this study was to develop novel, gluten-free licorice from quinoa and rice flours, and to evaluate the sensory and physical properties of the samples while comparing them to commercial gluten-free licorice. The licorice in this research was made with a twin-screw extruder. Samples were made in two batches, the first batch containing only quinoa licorice and the second batch containing both quinoa and rice licorice. In the first batch, the mass flow and the amount of anise aroma were the changing variables between the samples. With the second batch, two different flours (quinoa and rice) were used together with two different sugars (liquid sugar and coconut syrup) to get four different samples. The hypothesis for all samples in both batches was, that the changes in the production process or ingredients would lead to statistically significant changes in the sensory and physical properties of the licorice. The samples were studied with sensory methods as well as some mechanical tests, to establish the physical properties of the licorice. The sensory properties were evaluated with generic descriptive analysis by a trained panel and hedonic consumer tests with untrained panelists. Water activity was measured from the samples and physical properties were measured with extension and compression tests.

3.2 Materials and methods

3.2.1 Overview

This study is a part of bigger research concerning quinoa at University of Helsinki. Some research about quinoa has been published (Diaz et al., 2015; Rytkönen, 2017), a few other studies are ongoing. A couple of previous studies acted as the baseline for this research and helped define the parameters for extrusion, recipe and sensory evaluation procedures. The recipe was based on previous studies with wheat and quinoa licorice by Kallio (2006) and Rytkönen (2017). The extrusion parameters and the bases for the sensory evaluations were founded upon the results of the pre-tests. The flow chart in Figure 6 shows the experiments (including pre-tests) of the licorice studied in this research.

3.2.2 The pre-tests

The first pre-test (spring 2017) included four different samples, two of which were made from quinoa flour and the other two from wheat flour. This test focused on the differences between novel quinoa licorice and traditional wheat licorice. The samples varied between the used sugars as well and these two sugars (liquid sugar and coconut syrup) were the same types used in the actual study later. The aim of the first pre-test was to see how the consumers reacted to quinoa licorice in general, as this was the first time this kind of quinoa licorice was studied using sensory methods at the University of Helsinki. The pre-test revealed that consumers preferred the more traditional wheat licorice over the novel quinoa one. The panelists also preferred the samples with coconut syrup over the ones with liquid sugar, therefore coconut syrup was used for the most samples in the subsequent studies.

In the second pre-test (autumn 2017) three different samples were studied. One goal was to focus solely on gluten-free samples in this pre-test. All samples contained quinoa flour, but they had different aromas. The previously preferred coconut syrup was now used in all the samples as the main sugar. The aromas used were anise and tar, apart from the third sample, which had no additional aroma. This second test included a consumer test, and, in addition to the preference, the panelists also had to describe the different properties with a check-all-that-apply section. The anise-licorice was preferred over the others, and it was described to smell and taste most licorice-like. The tar one was described as overpowering and it divided people with a strong flavor while the sample with no additional aroma was described milder and blander than the anise-licorice. This second pre-test impacted on the amount of anise aroma used in further studies. The results from the pre-tests suggested that further development of the quinoa licorice was needed to increase its acceptability.

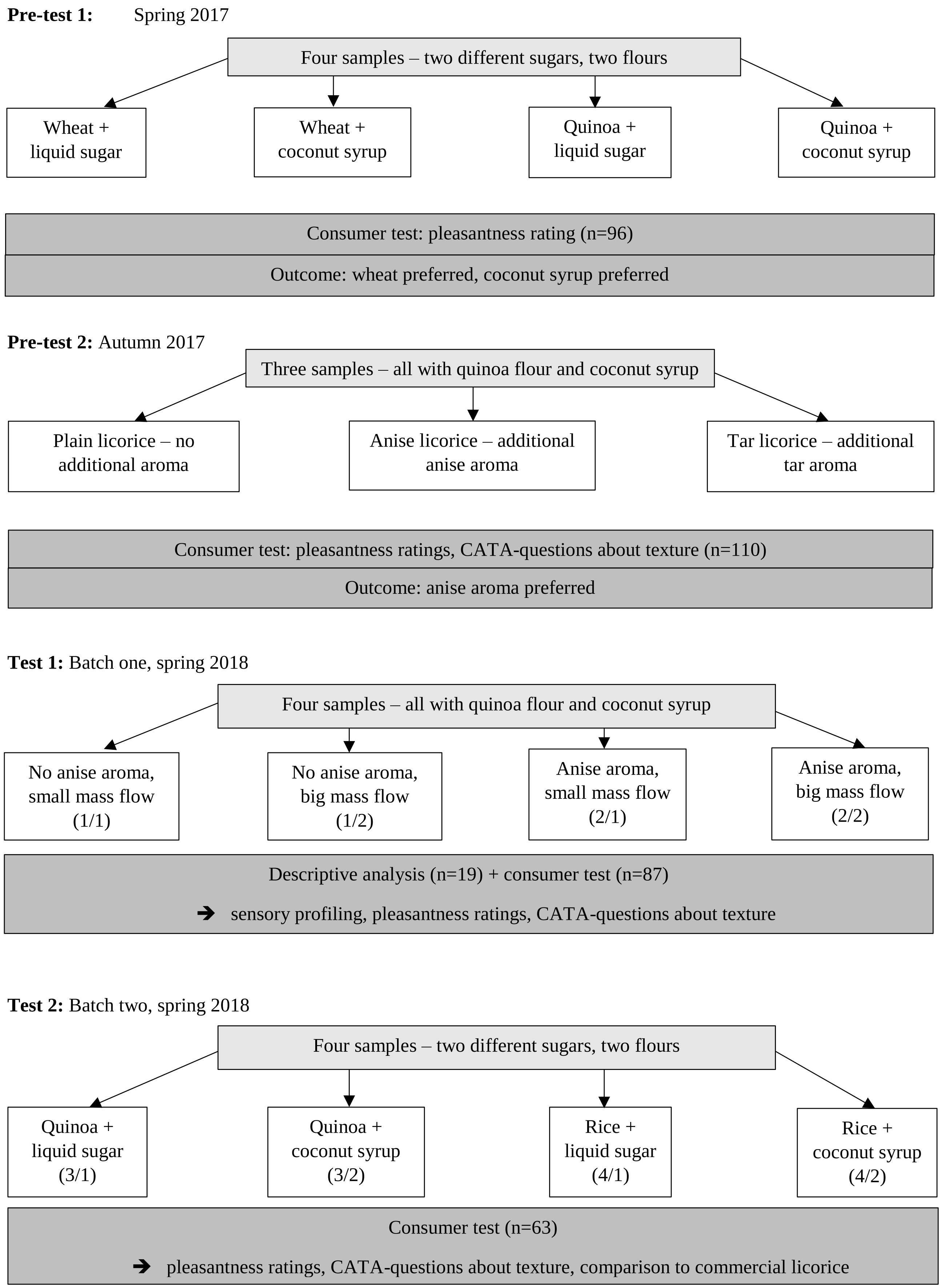


Figure 6. Flow chart of the sensory evaluations of quinoa licorice (pre-tests and this study)

These two pre-tests formed the baseline for this thesis research, in which two batches of licorice were studied (Figure 6). Here, in the first batch, it was decided, based on the results from the pre-tests, to make only quinoa licorice with coconut syrup. The varying parameters were focused on the extrusion process (different mass flows) as well as the amount of anise aroma in use. With this study, the comparison to commercial samples was also done. The commercial samples were made from rice flour and had a high amount of licorice extract in them (up to 6%). The presence of the commercial samples was the main reason for doing the second batch of licorice in this research. Both quinoa and rice licorice were manufactured because that enabled better comparison to the commercial samples. The high amount of licorice extract in the commercial samples was also the reason behind the increased amount of licorice extract in the samples of the second batch.

3.2.3 Ingredients

There were two different batches of gluten-free licorice made in this study. The first batch contained only quinoa licorice and the second batch contained both quinoa licorice and rice licorice. The first batch included coconut syrup, molasses, licorice extract, quinoa flour, salt, and water, plus anise oil and black food colorant (Table 2). The licorice made in the first batch was organic, since at least 95% of the ingredients were organic, which is the requirement for regarding a product as organic food (EVIRA, 2016).

Table 2. Ingredients used in the making of the licorice in this study

Ingredient	Manufacturer	Country of origin	Used in batch
Organic coconut syrup	Big Tree Farms	Indonesia	1 & 2
Liquid sugar	Nordic Sugar	Finland	2
Organic molasses	August Töpfer & Co	Germany	1
Molasses	Nordic Sugar	Finland	2
Quinoa flour	Raininko organic farm	Finland	1 & 2
Rice flour	Virtasalmen viljatuote Oy	Finland	2
Licorice extract powder	NATURE MED S.r.l.	Italy	1 & 2
Black colorant (E153)	Spice & Aroma Finland	Finland	1 & 2
Anise oil	Spice & Aroma Finland	Finland	1 & 2
Fine sea salt	Meira	Finland	1 & 2
Water	City of Helsinki water (tap)	Finland	1 & 2

With the second batch, also the sugars altered, resulting in licorice made either with liquid sugar or coconut syrup. In the second batch, the molasses was changed from the organic to non-organic, due to the mildly overpowering taste of the organic molasses used in batch number one. The second batch also contained two different licorice extracts, one was the same used in the first batch and the second was a fresh one (same manufacturer). For the extrusion, the two licorice extracts were mixed together beforehand, and the water content was measured as the average for the recipe. The second batch was not organic anymore due to all the changes.

3.2.4 Recipe

The two batches were made with slightly different recipes to achieve four different samples in both batches (total of eight). The basic recipe for both batches was already seen fit in previous research by Kallio (2006) and Rytkönen (2017) as well as in the two pre-tests. The recipe for the first batch was almost identical to those and the ratios can be seen in Table 3. Anise oil was only used in half of the samples in batch one, but other than that, the recipe is as shown in the table.

The second batch was made with the same basic recipe, except that the amount of licorice extract was raised up to 6%, which led to small changes in the ratios of other ingredients as well. The second batch also included either quinoa or rice flour as well as either liquid sugar or coconut syrup. Due to the different flour types and their slightly different water contents, the actual amounts of ingredients altered a little between the samples, but the overall percentages in the recipe were same with all the licorice samples in the second batch.

Table 3. Recipe for the licorices

Ingredient	Content of raw material (%)	
	Batch 1	Batch 2
Coconut syrup	49.8	46.4
Liquid sugar	-	46.4
Molasses	12.0	12.0
Licorice extract	2.6	6.0
Water	6.5	6.5
Salt	0.1	0.1
Quinoa flour	29.0	29.0
Rice flour	-	29.0
Black color	trace	trace
Anise oil	trace	trace

*with batch 2, only one sugar and one flour were used at the same time

The amounts of anise oil and black colorant (E153) were calculated with the help of the dosing instructions of the manufacturer (Spice&Aroma Finland, 2017) and the total mass of the licorice "dough". They are described as a trace in the recipe, due to the tiny amounts, but the actual amounts of aroma and colorant can be seen in Table 4.

Table 4. Amounts of anise oil and black colorant in the samples

Sample	Batch	Amount of anise oil (g)	Amount of colorant (g)
1/1	1	0.000	4.172
1/2	1	0.000	4.172
2/1	1	17.199	4.172
2/2	1	17.199	4.172
3/1	2	18.125	4.397
3/2	2	18.125	4.397
4/1	2	18.071	4.384
4/2	2	18.071	4.384

The water content of the licorice was set as 24 % in all the self-made samples. This needed to be balanced in the recipe and therefore the water contents of the solid ingredients had to be calculated. In this study, the specifications of the liquid ingredients, provided by the manufacturers, were trusted. The solid ingredients (flour, licorice extract) were weighed in metal containers before, put in heating cabinets and weighed again after the drying. Then the loss of weight was calculated into the water content percentage. The solid ingredients from the first batch were dried in vacuum heat cabinets (Salvis Vacucenter, 70 °C, 21 h). The second batch was made later in the study; here the solid ingredients were dried in a normal heat cabinet (Termarks, 130 °C, 1 h) according to the AACC-method 44-15.02 (AACC, 1981). The results of the water content measurements for both batches can be seen in Table 5 and the full calculations in Appendix 1.

Table 5. Water contents of dry ingredients

Sample	Water content (%)	Standard deviation
Quinoa (batch 1)	9.53	0.01
Licorice extract (batch 1)	7.31	0.01
Quinoa (batch 2)	9.91	0.11
Rice (batch 2)	10.54	0.22
Licorice extract (batch 2)	9.31	0.22

3.2.5 Samples

There were four licorice samples made in both batches. In the first batch, the mass flow and the amount of anise oil differed, in order to study potential differences in texture and flavor. In the second batch, the flour types and sugar types altered, again to study potential differences in the texture and flavor. All the eight different samples from both batches can be seen in Table 6.

Table 6. Licorice samples in this study

Batch	Variable 1	Variable 2	Sample
1	No anise oil	Smaller mass flow	1/1
1	No anise oil	Bigger mass flow	1/2
1	Anise oil	Smaller mass flow	2/1
1	Anise oil	Bigger mass flow	2/2
2	Quinoa flour	Liquid sugar	3/1
2	Quinoa flour	Coconut syrup	3/2
2	Rice flour	Liquid sugar	4/1
2	Rice flour	Coconut syrup	4/2

In addition to the self-made samples, there were also two commercial licorice samples that were used throughout this research. The commercial samples were gluten-free options from Porvoon lakritsi (Gluteeniton lakritsi) and Lakritsfabriken (Sweet Liquorice) and they were both made from rice flour. The commercial samples and their ingredients can be seen in Table 7.

Table 7. Commercial samples used in the study

Sample	Ingredients	Manufacturer
P	Sugar syrup, rice flour, cane sugar molasses, licorice extract, color (charcoal), natural aroma (anise oil)	Gluteeniton lakritsi, Porvoon lakritsi, Oy Walton's Ab
L	Cane sugar syrup, rice flour, cane sugar, invert sugar, licorice extract (6%), humectants: sorbitol, salt, anise oil; glazing agents: carnauba wax, vegetable oil (coconut oil)	Sweet Liquorice, Lakritsfabriken, Ramlösa

3.2.6 Extrusion

Experimental design

All the licorice was made at University of Helsinki, in EE-building (Viikki) at the process hall with a twin-screw extruder (TW24, Thermo Haake, PolyLab System, Germany). The solid ingredients were fed into the extruder’s first section, with the dosing screw (Brabender Technologie, DDSP20N-10Q, Germany). The liquids were mixed together in preparation and fed into the second section using a liquid pump (505S, Watson Marlow Limited, England). The extruder in use was the same throughout the process. The screws were rotating in the same direction in the extruder. The screws’ diameter was 24 cm and length 672 mm. The ratio between the length and the diameter (L/D) was 28. The screw speed was kept constant in all extrusions, at 55 rpm. The temperatures during extrusion can be seen in Figure 7, they were kept constant as well, with the help of Thermo Haake PolyLab System-program.

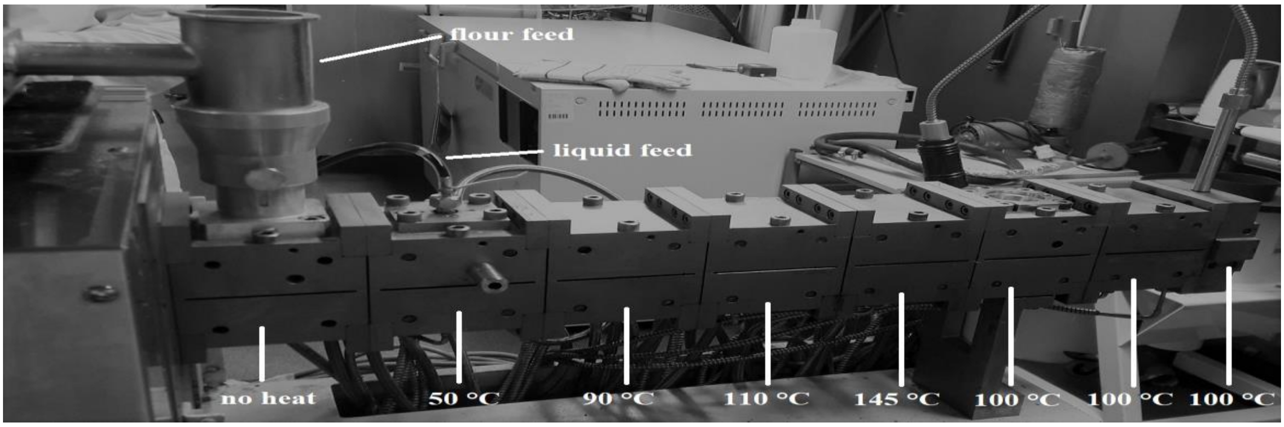


Figure 7. Temperatures in the extruder

Determining the mass flows

The goal with the first batch of licorice was to see potential differences in the structure, by altering the mass flow during the extrusion process. That is why the samples were made with two different mass flows. The mass flows were based on previous research by Rytkönen (2017) as well as the pre-tests, and therefore 80 g/min and 120 g/min were chosen. In the second batch, the mass flow was not one of the changing variables and therefore it was made with only one mass flow over the whole extrusion. The mass flow was chosen based on the results from the first batch and it was 100 g/min.

The mass flows had to be tested before the actual extrusion, so test drives were made for both batches by testing the feeding rates. Separate mass flows for the solid and the liquid mixtures were determined by feeding the mixtures in and weighing the outcome after one minute. The mass flows were determined with three different settings of feeding rates and each setting was tested three times for repetition. With the results of the feeding test drives, a figure could be drawn and from the equation, the mass flow could be calculated (Figure 8). The other equations can be seen in Appendix 2.

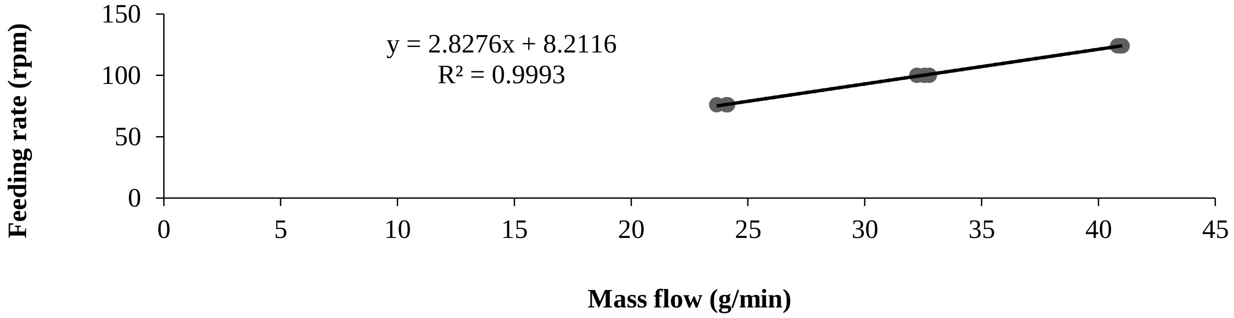


Figure 8. Mass flow of the solid mixture in first batch as the function of the feeding rate

The extrusion processes did not go quite as planned, and in both extrusions, the parameters had to be adjusted due to unexpected problems. The adjustment of feeding rates affected the final mass flows and therefore the re-calculated mass flows and feeding rates for both batches can be seen in Table 8.

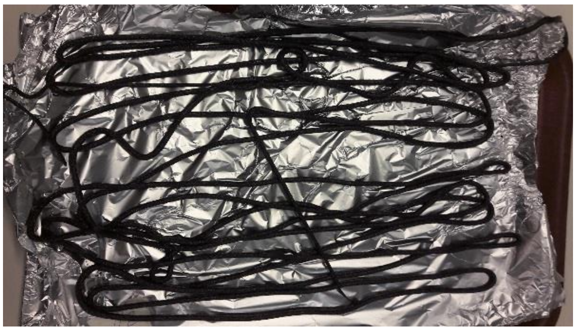
Table 8. The original and re-calculated mass flows and feeding rates in the extrusion

Sample	Original mass flow (g/min)	Actual mass flow (g/min)	Original flour feeding rate (rpm)	Actual flour feeding rate (rpm)	Original liquid feeding rate (rpm)	Actual liquid feeding rate (rpm)
1/1	80	100	82.4	100.9	15.4	19.3
1/2	120	130	119.5	128.8	23.2	25.1
2/1	80	100	119.5	128.8	23.2	25.1
2/2	120	130	82.4	100.9	15.4	19.3
3/1	100	100	103.8	103.8	52.9	52.9
3/2	100	100	103.8	103.8	18.4	18.4
4/1	100	100	116.0	218.0	52.9	13.1
4/2	100	100	116.0	218.0	18.4	9.0

Sampling

The licorice was collected on foil covered trays to dry for two hours in room temperature (Figure 9). There were at least eight trays of each sample collected for the evaluations. The licorice was cut into different sized pieces based on the use of it (sensory/instrumental analysis), and all the samples were kept in airtight bags until further testing.

A



B

Figure 9. Licorice samples collected on the tray to dry (A) and cut into small pieces for water activity determination (B)

3.2.7 Sensory evaluations

The samples were evaluated with two different sensory evaluation techniques. With the samples from the first batch, a generic descriptive analysis was conducted as well as a consumer test. With the samples from the second batch, only a consumer test was performed. In all sensory evaluations, two different commercial samples were included in the evaluation in addition to four self-made samples. All the sensory evaluations were performed at the Laboratory of Sensory Science in EE-building (Viikki), at University of Helsinki.

Generic descriptive analysis (GDA)

Descriptive analysis was used to evaluate analytically the different sensory properties of the samples on the first batch. The panel consisted of 19 judges, who were all food science students at the university and were attending the course “Advance Sensory Science (FOOD-116)” at the time. The panel was trained only in one session, but the panel was divided into two groups during training due to the larger size of the panel. The descriptions of the properties and the suitable scales for the evaluation were decided on, in the training session. The actual evaluation was then done twice in two consecutive days to get repeated ratings.

In the training session, three samples were used for panel training. Two out of three were different self-made samples and the third one was a commercial sample. At the training session, there was an additional reference sample that was used to help with the determination of appearance terminology (color, shine) of the evaluated samples. This reference sample was not used in the evaluation sessions. All the samples used in the actual evaluations are shown in Table 9. In the actual evaluation and repetition all the samples were cut into ca. 2 cm long pieces and placed on to clear, small plastic cups with three-digit codes on the side and served out to the judges on a tray. In the two evaluations, all six samples were evaluated in randomized order in one session. The sessions were conducted individually in booths at room temperature under white lighting. Tap water and neutral snacks were provided for mouth rinsing between samples.

Table 9. Samples used in the descriptive analysis (batch 1 samples)

Sample	Variable 1	Variable 2
1/1	No anise oil	Smaller mass flow
1/2	No anise oil	Bigger mass flow
2/1	Anise oil	Smaller mass flow
2/2	Anise oil	Bigger mass flow
P	Commercial 1	Porvoon lakritsi
L	Commercial 2	Lakritsfabriken

The panel settled for ten different attributes that would best describe the samples and reveal possible differences between them. The attributes were evaluated on a line-scale, where 0 was labeled as the end where the attribute was mild or non-existent, and 10 as the end where the attribute was at the strongest. The evaluation was conducted with FIZZ-Network. The attributes used in the evaluations can be seen in Table 10 and the total evaluation forms can be seen in Appendix 3.

Table 10. Sensory attributes used for describing the samples in the descriptive analysis

Attribute	Label at 0	Label at 10
Homogeneity of appearance	Grainy	Smooth
Shine	Matt	Shiny
Overall intensity of odor	No odor	Strong odor
Sweet odor	No odor	Strong odor
Homogeneity of texture	Grainy	Smooth
Stickiness	Not at all sticky	Very sticky
Overall intensity of taste	Mild	Strong
Sweet taste	Mild	Strong
Licorice taste	Mild	Strong
Intensity of aftertaste	Mild	Strong

All different sensory attributes were evaluated from 0 to 10 and the scores were calculated as such into average scores for the descriptive attribute, and the means and standard deviations are shown in diagrams. Each attribute was analyzed with one-way variance analysis and Tukey's test. The whole descriptive analysis was also analyzed with three-way variance analysis, so that the interactions of different samples, panelists and sessions could be seen. A principal component analysis was also performed from the results to show the relations among the samples and attributes.

Consumer tests

The consumer tests for both batches were done with untrained panels, first one with 87 panelists and the second one with 63 panelists. Consent forms for the consumer tests and the background questionnaire used can be seen in Appendix 4 and 5. The panel was first asked some background information about their gender, age, consumption of sweets and licorice and the type of licorice they commonly use. In the actual evaluation, the judges rated their intensity of liking for each sample on a nine-point hedonic scale ranging from very unpleasant (1) to very pleasant (9).

There were separate questions for appearance, odor, taste, texture and the overall pleasantness of the samples. The judges also had to evaluate the texture of the samples with a check-all-that-apply section at the end of the session.

The licorice samples were cut into ca. 2 cm long pieces and placed on to small plastic cups with lids and three-digit codes on the top. Six samples were evaluated in randomized order in the session, and the session was conducted individually in booths at room temperature under white lighting. Tap water and neutral snacks were provided for mouth rinsing between samples as seen on the tray in Figure 10.

Figure 10. The tray ready for the sensory evaluation

The samples used in the consumer test can be seen in Table 11. A similar consumer test was conducted with both batches, the commercial samples being the same throughout the study.

Table 11. Samples in the consumer tests

Sample	Variable 1	Variable 2	Test
1/1	No anise oil	Smaller mass flow	1
1/2	No anise oil	Bigger mass flow	1
2/1	Anise oil	Smaller mass flow	1
2/2	Anise oil	Bigger mass flow	1
3/1	Quinoa flour	Liquid sugar	2
3/2	Quinoa flour	Coconut syrup	2
4/1	Rice flour	Liquid sugar	2
4/2	Rice flour	Coconut syrup	2
P	Commercial 1	Porvoon lakritsi	1 & 2
L	Commercial 2	Lakritsfabriken	1 & 2

The check-all-that-apply questions were used at the end of the evaluation, and the judges had to describe the texture of the samples in more detail. These attributes were the same as used in one of the pre-tests (Table 12) and they represent different textures of typical licorice, which is why they were used in the first place.

Table 12. The attributes in the check-all-that-apply section in the consumer tests

Codes	Attributes
A1	Soft
A2	Hard
A3	Chewy
A4	Sticky
A5	Smooth
A6	Rubbery
A7	Cleaved
A8	Dry
A9	Grainy

Pleasantness scores were quantified with scores from 1 to 9, one being very unpleasant and nine being very pleasant. The check-all-that-apply-questions were the same in both consumer tests and the results were demonstrated with a radar-chart. The average scores and standard deviations were calculated. The pleasantness was also studied with one-way analysis of variance and Tukey's test. The evaluation form used for the consumer test can be seen in Appendix 6.

3.2.8 Other analyses

In addition to the sensory evaluations, there were some tests performed to measure the properties of the samples: water activity and mechanical tests. The analyses were only conducted with the self-made and commercial samples of the first batch and can be seen in full form in Appendix 11. Water activity was determined with both Novasina Labmaster (Novasina AG, Switzerland) and AquaLab (METER Group Inc, USA) equipment, the temperature was 20 °C. The samples were cut into tiny pieces, 20 pieces always acted as one testing sample, and placed on small plastic cups. There were two replicates from each sample and one sample was measured for 30 minutes at a time simultaneously with the two equipment. The average water activity was calculated for each sample.

The mechanical tests included extension and compression tests. The tests were done with a universal testing machine (Instron 4465, UK) using existing software to calculate the values of extension and compression. The extension test (Figure 11) was done only for the four self-made samples to compare them to previous researches since the commercial licorice samples were too small to handle the test (10 cm long pieces needed). The extension was tested with 10 replicates from each sample. The sample was adjusted into the roll-shaped coupling heads 7 cm apart from each other and the pulling speed was set at 10 mm/min. The tensile strength was calculated from the average extensions, dividing the force at maximum load (N) by the displacement at the maximum load (mm) to gain the tensile strength (N/mm).

Figure 11. Extension test

The compression test could be done to both the self-made samples and the commercial ones. The samples were cut into 1.5 cm long pieces pushed together with a square plack with 3 mm/min speed. The compression test was also done with 10 replicates for better accuracy. The compressive strength was calculated dividing the primary force (N) by the compressive displacement (mm) to gain the compressive strength (N/mm).

3.3 Results

3.3.1. Sensory characteristics

The descriptive analysis was done with the ten descriptive attributes that the trained panel (n=19) chose in the training session (Table 10). They had two descriptive attributes for appearance, odor and texture each and four attributes describing the taste. From the results of the general descriptive analysis, a sensory profile of the samples was made. The radar-chart made from the results is shown in Figure 12. In almost all the descriptive attributes, the commercial samples got higher values than the self-made samples. No significant differences were observed in overall intensity of odor and stickiness. With the homogeneity of texture and appearance, the differences were the greatest between the self-made and commercial samples. Significance between groups from the one-way analysis of variance can be seen in the figure with the significance marks. The results of the full analysis of variance can be seen in Appendix 7.

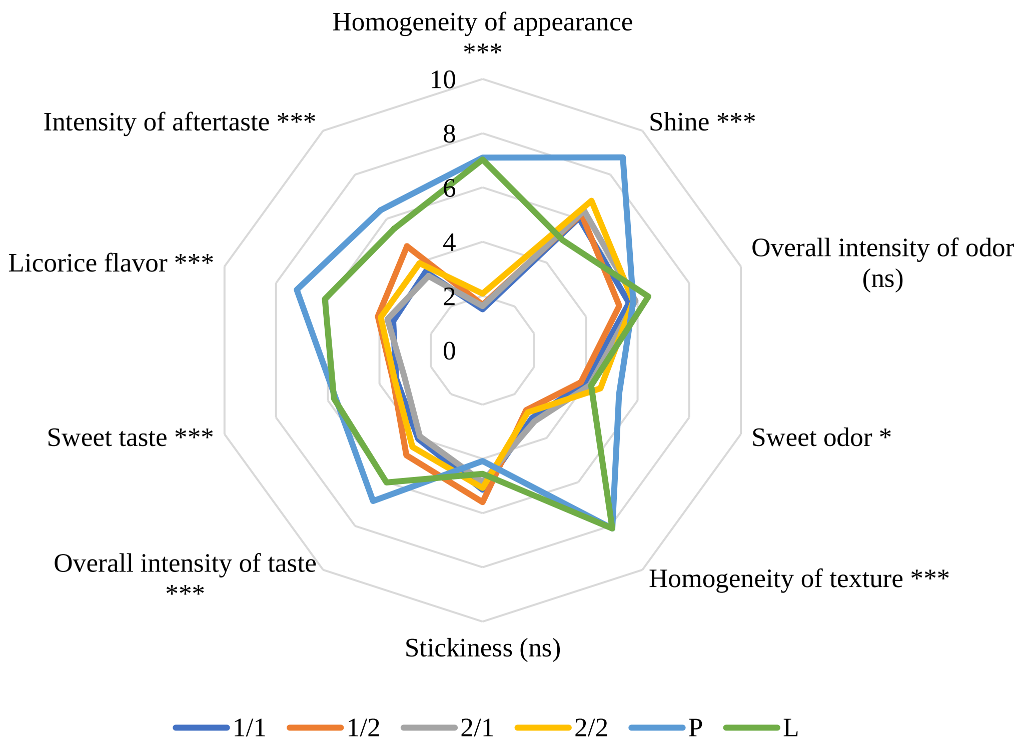


Figure 12. The sensory profiles of the licorice samples (Table 9) by GDA (Tukey's test ***, p<0.001; ns, not significant; n=19 panelists; the samples were evaluated twice)

Appearance

The appearance of the samples was described first, with two different attributes, homogeneity of appearance and shine, see Table 9 for details about the samples. The means and standard deviations for both attributes are shown in Figure 13. The commercial licorice samples were described as more homogenous in their appearance than the self-made samples (Figure 13A). Tukey's test confirmed the result, by grouping the self-made samples into one group (a) and the commercial samples into another (b).

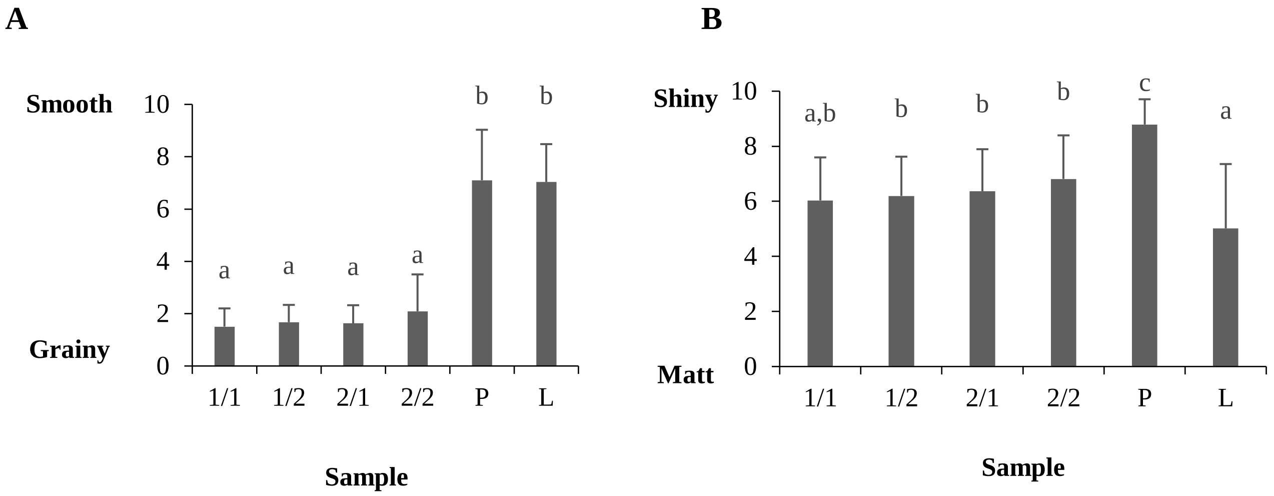


Figure 13. The means and standard deviations of homogeneity of appearance (A) and shine (B) of the licorice samples (Table 9) by GDA; means marked by different letters differ significantly (Tukey's test: $p<0.05$; n=19 panelists; the samples were evaluated twice)

The commercial sample from Lakritsfabriken (L) was described as the least shiny of all the samples, while the other commercial sample from Porvoon lakritsi (P) was described as the shiniest (Figure 13B). Tukey's test revealed that with the shine, all the self-made samples belonged to one group (b) and that the shiniest sample P had its own group (c). The self-made sample with no anise and small mass flow (1/1) was paired together with the commercial sample L in the same group (a).

Odor

The next two attributes focused on the odor of the samples and the panelist had to rate the overall intensity and the intensity of the sweet odor. The means and standard deviations for odor descriptions shown in Figure 14. The average overall intensity of odor of the samples did not vary greatly between the samples (Figure 14A). Nominally, the commercial sample from Lakritsfabriken had the most intense odor and self-made sample (1/2) with no anise and the bigger mass flow, had the least intense odor. However, Tukey's test stated, that all the samples belonged to the same group with the overall intensity of odor and that there were no significant differences between the samples.

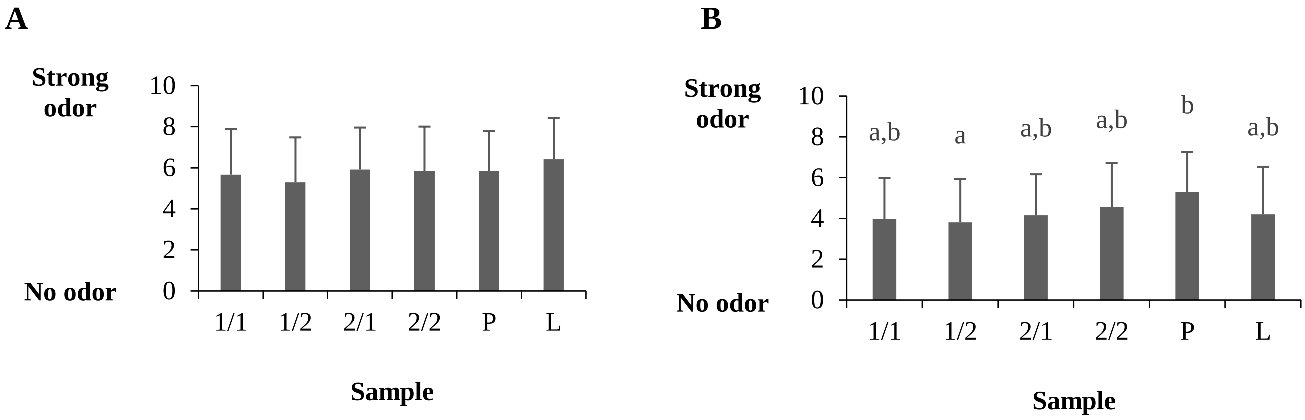


Figure 14. The means and standard deviations of overall intensity of odor (A) and sweet odor (B) of the licorice samples (Table 9) by GDA; means marked by different letters differ significantly (Tukey's test: $p<0.05$; n=19 panelists; the samples were evaluated twice)

Describing the sweetness of the odor was not that unanimous anymore (Figure 14B). The commercial sample from Porvoon lakritsi (P) was evaluated with the sweetest odor at 5.3, while sample (1/2) with no anise and the bigger mass flow was described as the sample with the least sweet odor. Tukey's test divided the samples into two groups so that the samples 1/2 and P can be seen was observed differing from one another.

Texture

Then the panel moved on describing the texture. Here in Figure 15, we can see the means and standard deviations of the homogeneity of the texture as well as the stickiness.

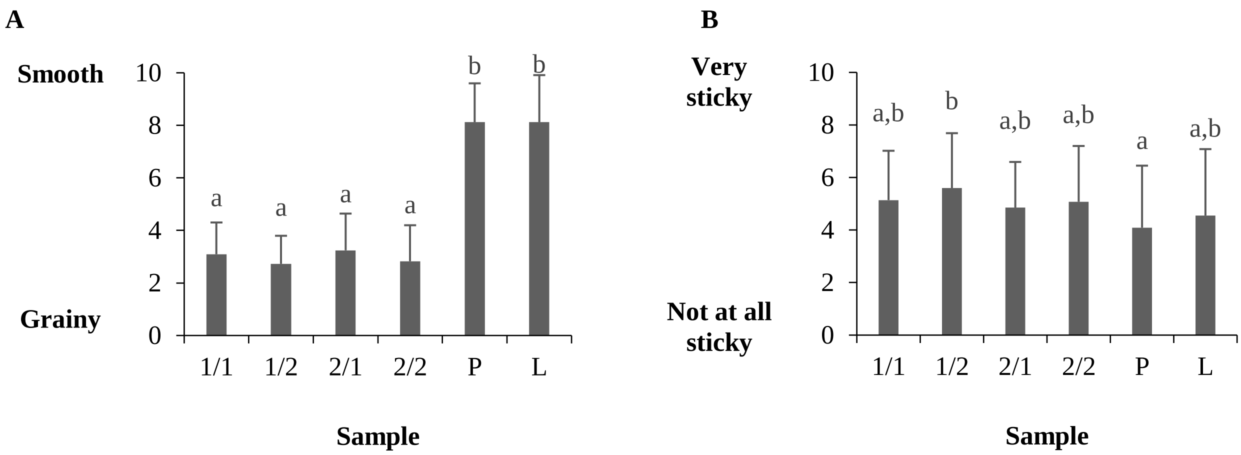


Figure 15. The means and standard deviations of homogeneity of texture (A) and stickiness (B) of the licorice samples (Table 9) by GDA; means marked by different letters differ significantly (Tukey's test: $p<0.05$; n=19 panelists; the samples were evaluated twice)

The same kind of pattern can be seen in the homogeneity of texture, as with the homogeneity of appearance, the commercial samples being described more homogenous than the self-made samples (Figure 15A). Based on the Tukey's test we can see the same direction of grouping, the self-made samples in a group (a) and the commercial samples in a group (b). The commercial sample P was described at least sticky at 4.1 and sample (1/2), with no anise and bigger mass flow, as the stickiest at 5.6 (Figure 15B). There were no big differences between any of the samples in the Tukey's test, however, samples 1/2 and P differed again slightly but significantly nevertheless, resulting in two groups.

Taste

The panel had to evaluate the taste of the samples with four different attributes. The first one was the overall intensity of the taste and its results are shown in Figure 16. Here, the commercial sample from Porvoon lakritsi was described as the sample with the most intense taste (6.9), followed by the other commercial sample from Lakritsfabriken (6,0). The self-made samples got notably lower values as the commercial samples at 3.9-4.8. Tukey's test showed that the self-made samples are grouped again in one group (a) and the commercial samples in another (b).

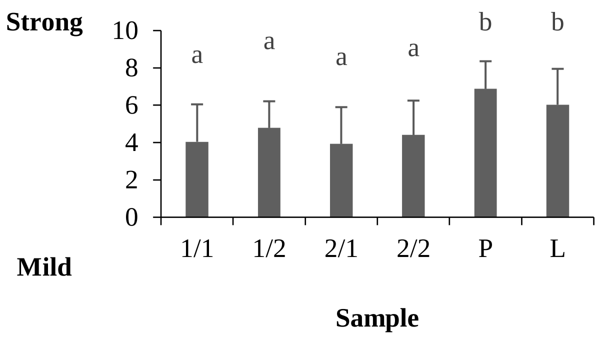


Figure 16. The means and standard deviations of the overall intensity of taste of the licorice samples (Table 9) by GDA; means marked by different letters differ significantly (Tukey's test: $p<0.05$; n=19 panelists; the samples were evaluated twice)

The sweet taste and the licorice taste (flavor) of the samples were also evaluated (Figure 17). The commercial sample L got the highest average, closely followed by the sample P (Figure 17A). The results of Tukey's test showed that the self-made samples differ from the commercial samples significantly, but not from each other. The commercial sample from Porvoon lakritsi was described as having the most intense licorice taste out of all the samples at 7.2 (Figure 17B).

From the self-made samples, the sample with no anise and a smaller mass flow (1/1) was evaluated as having the least licorice-like taste. With the licorice taste, the variance analysis grouped the self-made samples together and significantly different from the commercial samples.

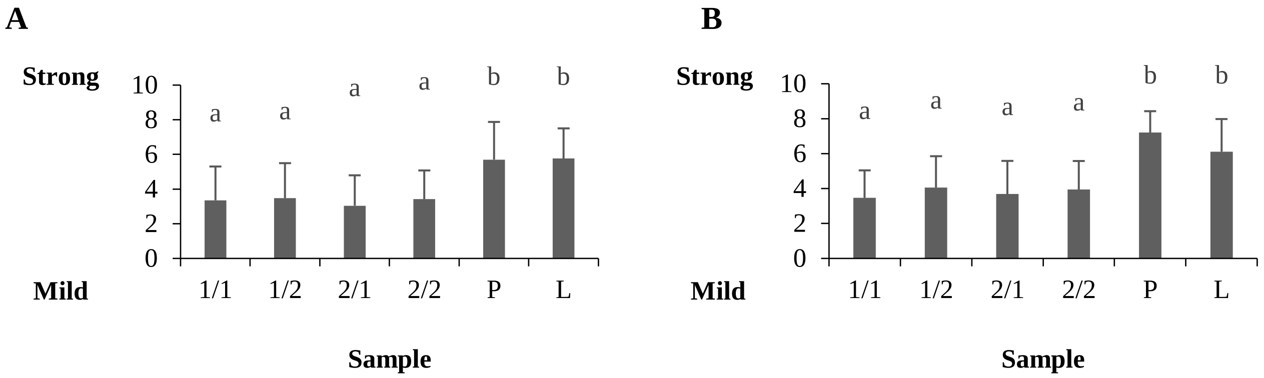


Figure 17. The means and standard deviations of the sweet taste (A) and the licorice taste (B) of the licorice samples (Table 9) by GDA; means marked by different letters differ significantly (Tukey's test: $p<0.05$; n=19 panelists; the samples were evaluated twice)

The last attribute in the evaluation that was rated, was the intensity of the aftertaste. The results can be seen in Figure 18. The commercial sample from Porvoon lakritsi (P) had the most intense aftertaste and the self-made sample with anise and the smaller mass flow (2/1) had the least intense aftertaste. The self-made samples 1/2 and 2/1 differed from each other in the Tukey's test. The self-made samples differed again from the commercial samples, except sample 1/2 from sample L. The commercial samples did not differ significantly from each other.

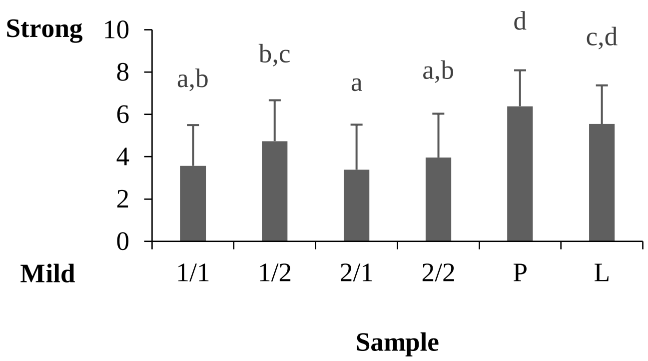


Figure 18. The means and standard deviations of the intensity of aftertaste of the licorice samples (Table 9) by GDA; means marked by different letters differ significantly (Tukey's test: $p<0.05$; n=19 panelists; the samples were evaluated twice)

Three-way analysis of variance

With the descriptive analysis also a three-way analysis of variance was conducted. The results can be seen in Table 13. It can be seen, that there were significant differences between the samples in all attributes, except the overall intensity of odor, as could be seen from the previous results of the descriptive analysis. In the second row, the results for the two sessions can be seen, and there were no significant differences in the results between the sessions.

Table 13. Three-way analysis of variance from the descriptive analysis

Interaction	Homogeneity of appearance	Shine	Overall intensity of odor	Sweet odor	Homogeneity of texture	Stickiness	Overall intensity of taste	Sweet taste	Licorice taste	Intensity of aftertaste
Sample	<0.001	<0.001	0.499	0.015	<0.001	0.041	<0.001	<0.001	<0.001	<0.001
Session	0.933	0.415	0.674	0.797	0.920	0.965	0.519	0.030	-	0.549
Panelist	0.005	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.001
Sample*Session	0.847	0.173	0.038	0.646	0.081	0.712	0.298	0.713	0.905	0.631
Sample*Panelist	0.368	<0.001	0.011	0.002	<0.001	0.011	0.001	<0.001	0.003	0.049
Session*Panelist	0.788	0.035	0.001	0.081	0.016	0.727	0.498	0.067	0.818	0.316

From the third row, the result for the panelists can be seen. There were significant differences by different panelists in all attributes. The interaction with sample and session was significant just with the overall intensity of the odor, but not with any other attribute. The interaction with sample and panelist was significant in most attributes, only with the homogeneity of appearance the difference was not significant. In the last row can be seen the results for the interaction between session and panelist. Here most of the attributes were not described significantly different.

Principal component analysis (PCA)

A principal component analysis (PCA) was conducted from the data of the generic descriptive analysis. The results are shown in Figure 19. The additional figures of the principal component analysis can be seen in Appendix 8. From this figure here, it can be seen, that the principal component one (PC-1) explains 74% of the interactions between the samples and the attributes. Principal component two (PC-2) then explains most of the remaining interactions at 16%. The third principal component explains the remaining 8% of the interactions, but since the principal component 1, explains most of the interactions the third one is not that significant.

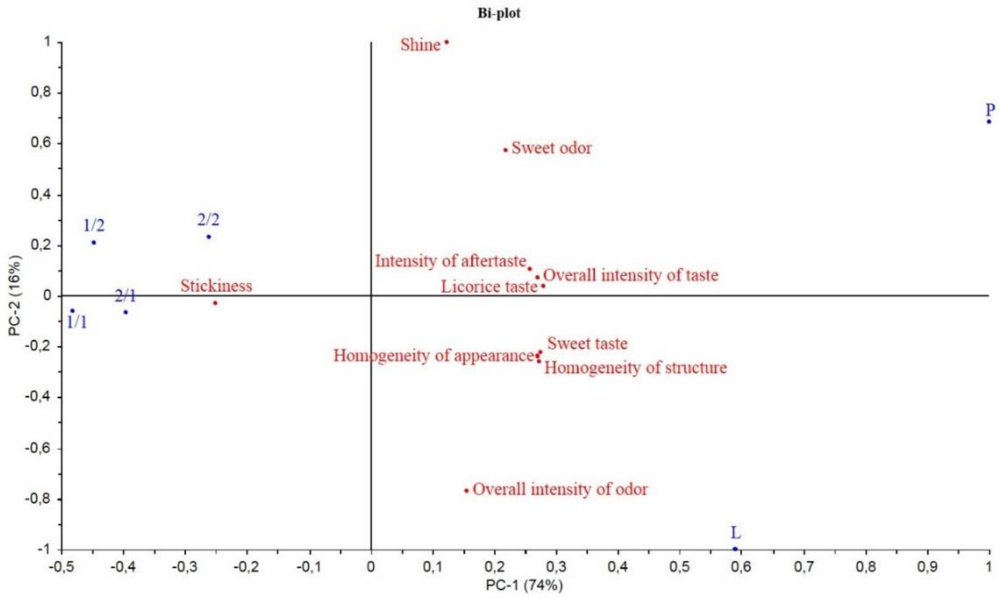


Figure 19. Principal component analysis with principal components one and two (n=19)

3.3.2 Consumer test with first batch

There were two separate consumer tests conducted with the licorice samples. The first one was done with the samples from the first batch and the two commercial samples (see Table 11). The results from the first consumer test are presented below.

Background information

The panel in the first consumer test consisted out of 87 people. There were 73 female and 14 male panelists present. The average age of the panelists was 30 years, the youngest panelist being 18 and the oldest 70 years old. In the background survey, the panelists were asked about their consumption of sweets and licorice, as well as the types of licorice they usually consume. The answers can be seen in Figures 20 and 21.

67% of the panelists ate sweets at least once a week or more frequently. In addition, almost 26% ate sweets a couple of times a month so it can be stated that the consumption of sweets was high among the panelists. Compared to the licorice consumption it can be seen, that 37% of the panelists ate licorice couple of times a month and nearly 24% ate licorice just once a month, so the consumption of licorice is less frequent than the overall consumption of sweets and, in addition, 30% of the panelists also only ate licorice a couple of times a year or less.

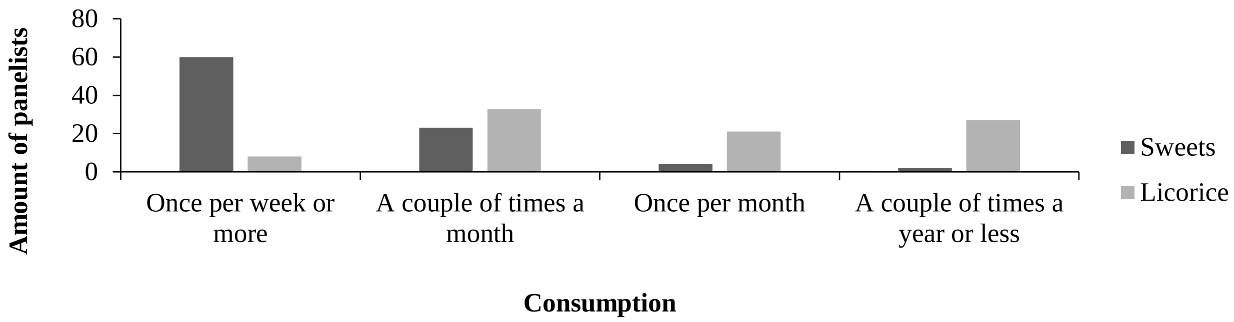


Figure 20. First consumer tests panelists consumption of sweets and licorice (n = 87)

The panelists also had to describe the type of licorice they usually eat. Plain licorice and filled licorice were most popular types with 29% and 21%. Also, salmiac licorice and coated licorice got higher averages as well, 15% each.

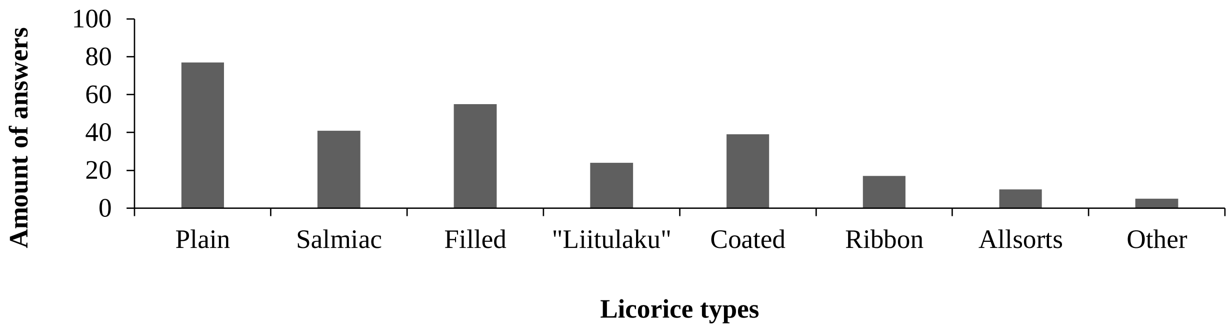


Figure 21. First consumer tests panelists consumption of different licorice types (n = 87)

Pleasantness ratings

In the actual evaluation, the panelists had to evaluate the pleasantness of appearance, odor, taste, and texture as well as the overall pleasantness of the licorice samples. The results for the pleasantness of the appearance are shown in Figure 22 and the full analysis of variance in Appendix 9. The commercial sample from Porvoon lakritsi was evaluated as the most pleasant in appearance with an average of 7.8. The self-made samples were rated with notably lower averages and the commercial sample from Lakritsfabriken was evaluated in the middle. The self-made sample with anise and small mass flow (2/1) was the least pleasant in appearance. The one-way analysis of variance and Tukey's test showed that the differences between self-made samples and commercial samples were significant. The two commercial samples also differed from each other creating three different groups, since the difference between the samples was significant.

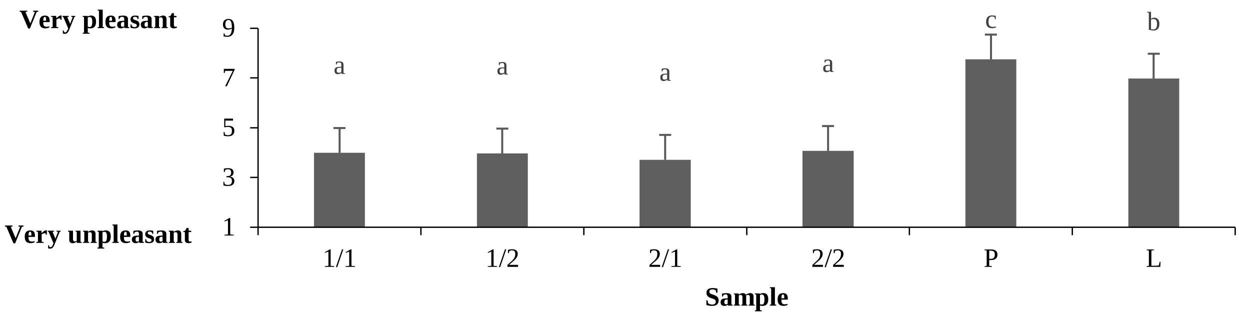


Figure 22. Pleasantness of appearance of the licorice samples (Table 11) in the first consumer test; means marked by different letters differ significantly (Tukey's test $p<0.05$ $n = 87$ panelists)

Next up the panel had to evaluate the pleasantness of odor and in Figure 23 the means and standard deviations are shown. Commercial sample from Porvoon lakritsi was evaluated with the most pleasant odor with an average of 6.5, while the sample with no anise and big mass flow (1/2) was considered least pleasing. The self-made samples were paired in two different groups by Tukey's test and sample L was found similar with both groups, while sample P was found significantly different from the self-made samples that did not contain anise (1/1 and 1/2).

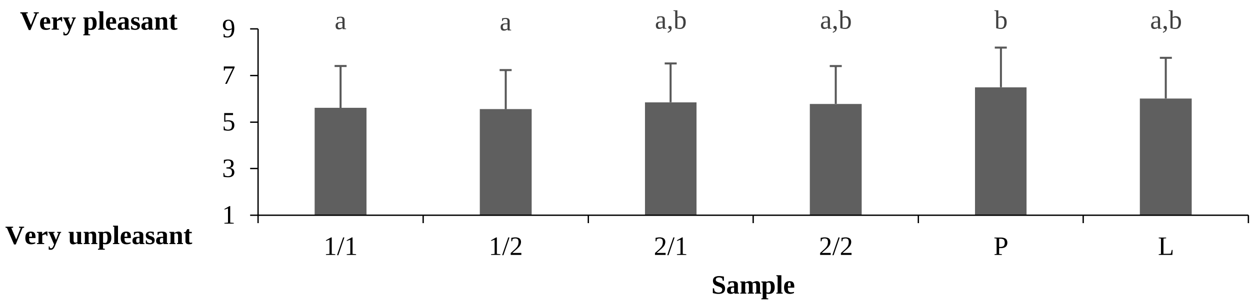


Figure 23. Pleasantness of odor of the licorice samples (Table 11) in the first consumer test; means marked by different letters differ significantly (Tukey's test $p<0.05$ $n = 87$ panelists)

The means and standard deviations of the pleasantness of the taste can be seen in Figure 24. Here the most pleasant samples were the commercial samples with averages of 6.8 and 6.6. The one-way variance analysis stated that the differences between groups were significant, so all the self-made samples differed significantly from the commercial samples, but not from each other. Tukey's test divided the samples into two groups, self-made samples in one (a) and the commercial samples in another (b).

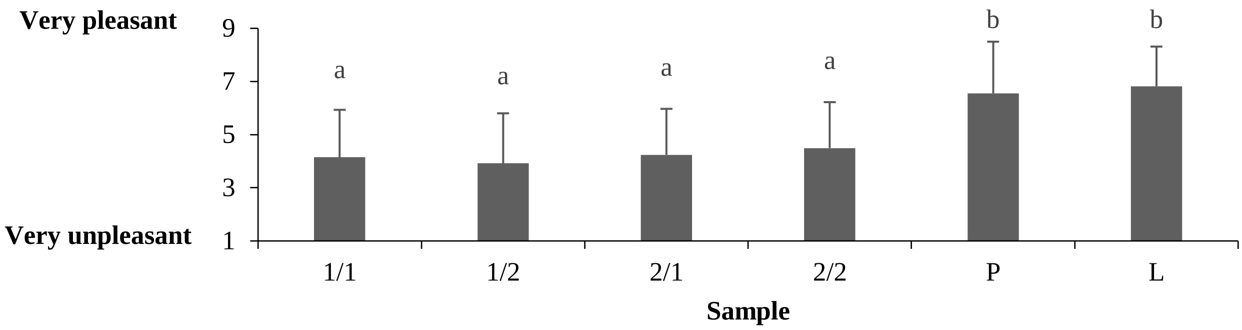


Figure 24. Pleasantness of taste of the licorice samples (Table 11) in the first consumer test; means marked by different letters differ significantly (Tukey's test p<0.05 n = 87 panelists)

Second to last pleasantness evaluation focused on the texture and its results can be seen in Figure 25. The most pleasant sample was the commercial sample from Porvoon lakritsi with an average of 7.0 and the self-made sample with no anise and the bigger mass flow (1/2) was ranked least pleasing in texture at 4.1. The results of the one-way variance analysis showed that there were significant differences between the groups and based on the Tukey's test the self-made samples formed one group (a) and the commercial samples another (b).

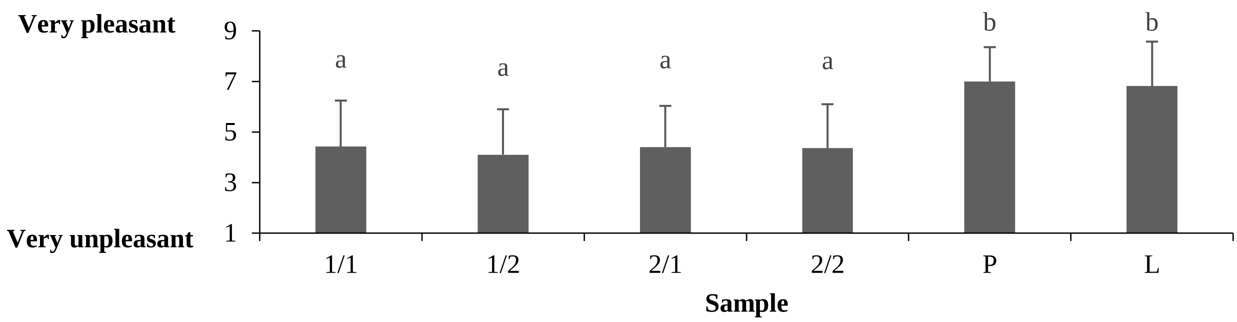


Figure 25. Pleasantness of texture of the licorice samples (Table 11) in the first consumer test; means marked by different letters differ significantly (Tukey's test p<0.05 n = 87 panelists)

The last pleasantness rating was the evaluation of the overall pleasantness. The means can be seen in Figure 26. The commercial sample from Lakritsfabriken got the highest average at 7.4, followed by the other commercial sample. The one-way analysis of variance stated that there were significant differences between the groups, but they are only showing between the self-made samples when compared to the commercial samples. Tukey's test put the samples into two groups, self-made samples in one (a) and the commercial samples in another (b).

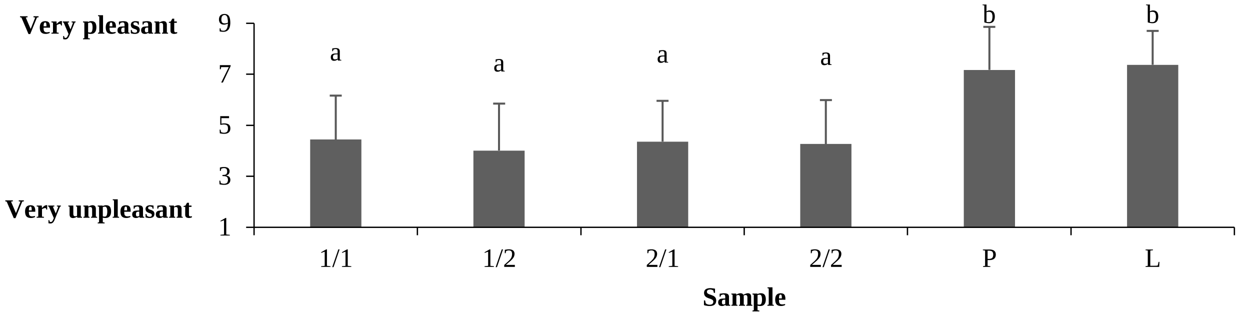


Figure 26. Overall pleasantness of the licorice samples (Table 11) in the first consumer test; means marked by different letters differ significantly (Tukey's test p<0.05 n = 87 panelists)

Check-all-that-apply evaluation

The session ended in a quick section with check-all-that-apply questions, where the panelists had to describe the texture of the samples in more detail. Figure 27 shows the results, from which it can be seen, that all the self-made samples follow similar lines in all attributes and get higher marks in grainy and dry texture. The commercial sample from Porvoon lakritsi is described as smooth, but cleaved and harder than the others, while the sample from Lakritsfabriken is described as smooth, soft and chewy.

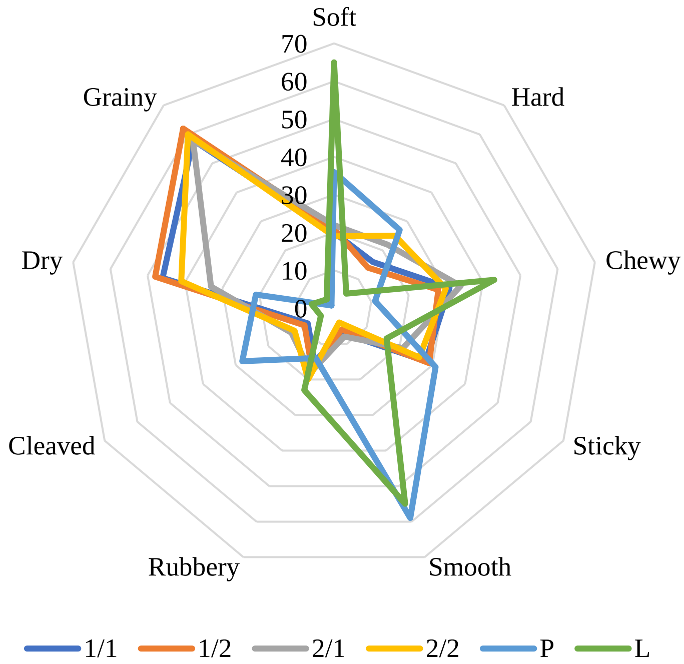


Figure 27. Results of the CATA-questions for the texture of the licorice samples (Table 11) in the first consumer test (n=87, results as amount of answers)

3.3.3 Consumer test with second batch

The second consumer test was done with the samples from the second batch and with the same two commercial samples that were used in the descriptive analysis and the first consumer test (see Table 11). The results from the second consumer test are presented below.

Background information

The panel in the second consumer test consisted of 63 people. The same type of basic background information was collected from the panelists than in the first consumer test. There were 53 female and 10 male panelists present. The average age of the panelists was 31 years, the youngest panelist being 19 years old and the oldest 66 years old. In the background survey, the panelists were asked about their consumption of sweets and licorice, as well as the type of licorice consumed (Figure 28).

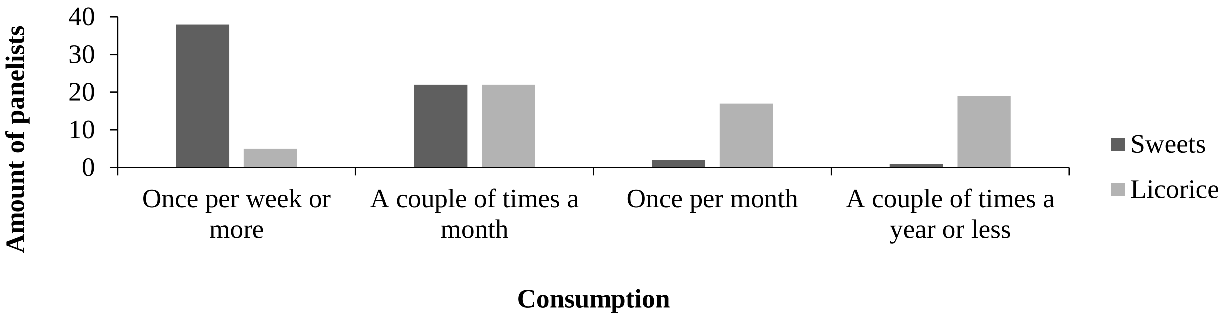


Figure 28. Second consumer test panelists consumption of sweets and licorice (n = 63)

60% of the panelists ate sweets at least once a week or more frequently. Also, 35% of the panel ate sweets a couple of times a month, so again the consumption of sweets is quite high. 35% of the panelists, however, ate licorice couple of times a month and nearly 27% ate licorice just once a month, so the consumption of licorice is again less frequent than the overall consumption of sweets. Considering that 30% of the panelists only ate licorice once a year or less frequently, the consumption of licorice varies a lot between the panelists. The panelists also had to describe the type of licorice they usually eat. The means can be seen in Figure 29. Plain licorice and filled licorice were most popular types with 26% and 22%. Salmiac licorice was fairly popular as well with 18%.

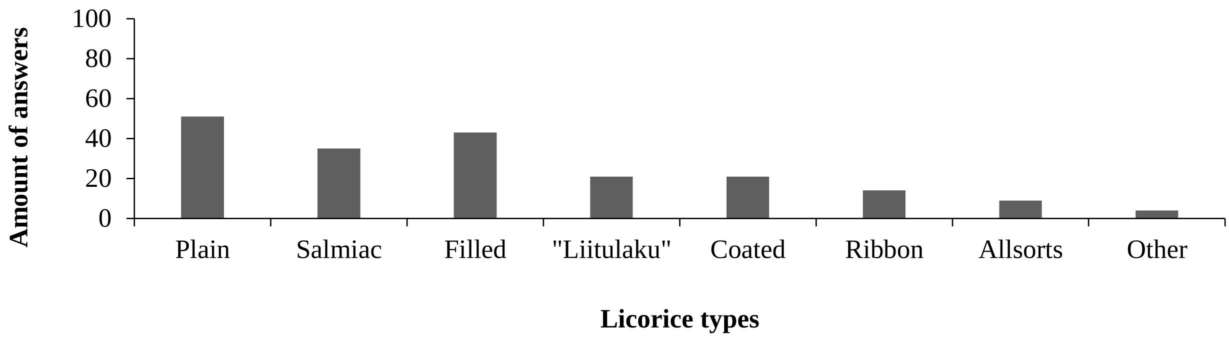


Figure 29. Second consumer tests panelists consumption of different licorice types (n = 63)

Pleasantness ratings

The means of the pleasantness of appearance can be seen in Figure 30 and the full analysis of variance in Appendix 10. Porvoon lakritsi (P) was evaluated as the most pleasant in appearance with an average of 7.9. According to the results of the one-way analysis of variance, the samples had significant differences between groups. The self-made samples were significantly different from the commercial samples, but samples 3/2 and 4/1 were also significantly different from each other, the rice sample 4/1 preferred over the quinoa sample. The two commercial samples were significantly different from each other as well.

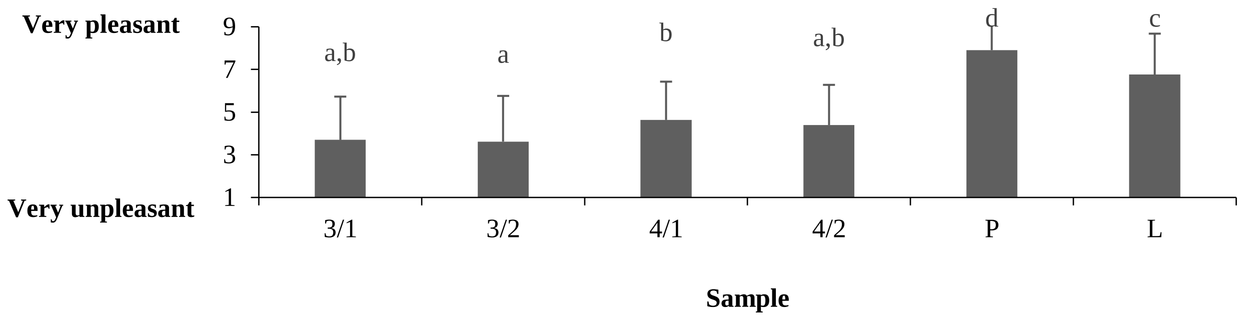


Figure 30. Pleasantness of appearance of the licorice samples (Table 11) in the second consumer test; means marked by different letters differ significantly (Tukey’s test $p<0.05$ n = 63 panelists)

The pleasantness of odor was evaluated after the appearance (Figure 31). All self-made samples were evaluated similarly in one group (a) by Tukey’s test, together with the commercial sample from Lakritsfabriken. The self-made samples were also seen similar to the other commercial sample P in another group (b). Sample P got the highest average at 6.4.

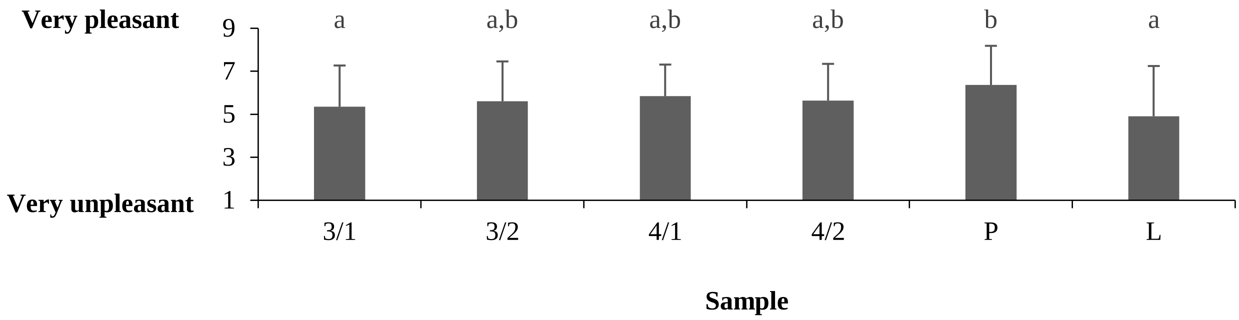


Figure 31. Pleasantness of odor of the licorice samples (Table 11) in the second consumer test; means marked by different letters differ significantly (Tukey's test $p<0.05$ $n = 63$ panelists)

The pleasantness of taste was evaluated next and the means and standard deviations can be seen in Figure 32. Now the most pleasant samples were the two commercial samples (6.7-6.8). From the self-made samples, the quinoa ones were evaluated as more pleasing than the rice samples. Tukey's test divided the sample into three groups, the commercial samples belonging to one (c), rice samples to another (a) and the quinoa samples to the third (b), although the rice sample with liquid sugar belonged also to a group (b).

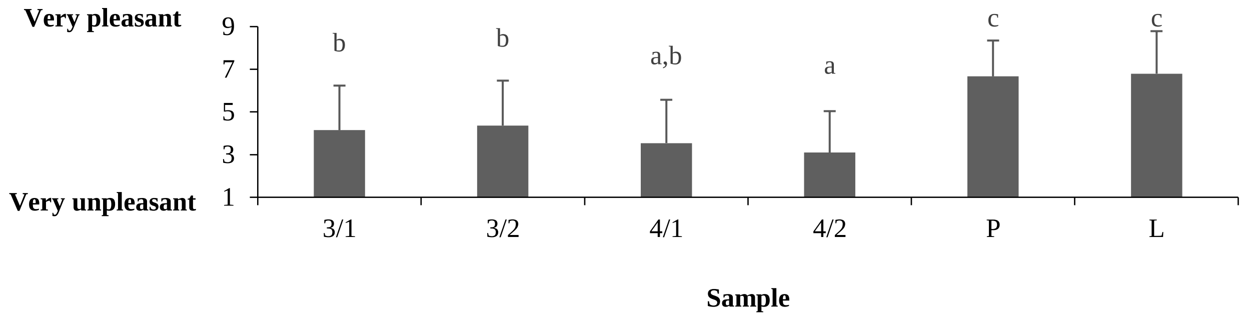


Figure 32. Pleasantness of taste of the licorice samples (Table 11) in the second consumer test; means marked by different letters differ significantly (Tukey's test $p<0.05$ $n = 63$ panelists)

The results for the pleasantness of texture can be seen in Figure 33. The most pleasant sample was the sample from Porvoon lakritsi with an average of 7.0. From the self-made samples, the quinoa licorice samples were better liked than the rice samples. The samples were divided into three groups with the Tukey's test. The one-way variance analysis showed significance between groups and stated that sample 3/2 (quinoa, coconut sugar) was significantly different from the rice samples 4/1 and 4/2. All the self-made samples were also significantly different from the commercial samples, but the commercial samples were not significantly different from each other.

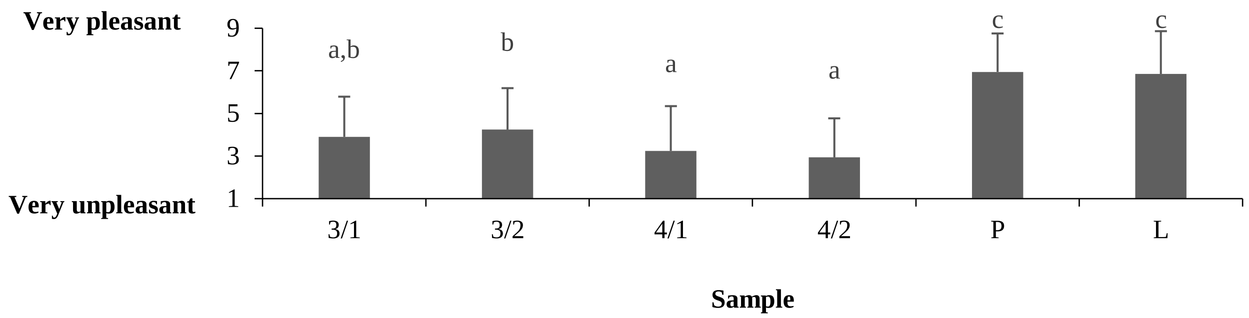


Figure 33. Pleasantness of texture of the licorice samples (Table 11) in the second consumer test; means marked by different letters differ significantly (Tukey's test p<0.05 n = 63 panelists)

The last attribute in the evaluation was the overall pleasantness of the samples and the results can be seen in Figure 34. The commercial samples got better averages than the self-made samples at 7.1-7.3 Quinoa licorice performed better than rice licorice from the self-made samples. These results were significantly different between groups, and there were three different groups of samples according to the Tukey's test. All self-made samples differed from the commercial ones and self-made samples 3/2 and 4/2 (quinoa/coconut sugar & rice/coconut sugar) were found significantly different as well.

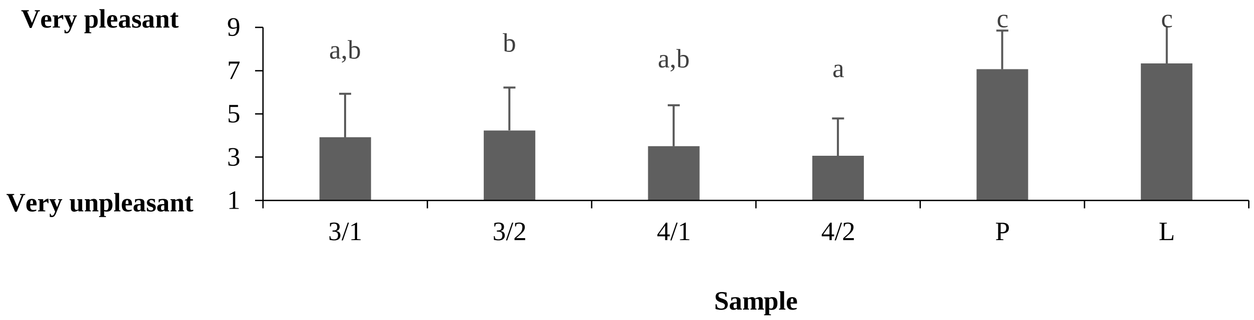


Figure 34. Overall pleasantness of the licorice samples (Table 11) in the second consumer test; means marked by different letters differ significantly (Tukey's test p<0.05 n = 63 panelists)

Check-all-that-apply evaluation

The evaluation ended again in a check-all-that-apply-section, where the panelists had to describe the texture of the samples. Figure 35 shows the results, from which it can be seen, that the self-made samples followed the same lines in two separate groups. The quinoa samples were described as grainy and chewy, while the rice samples were dry, hard and grainy, and the commercial samples were described as soft and smooth.

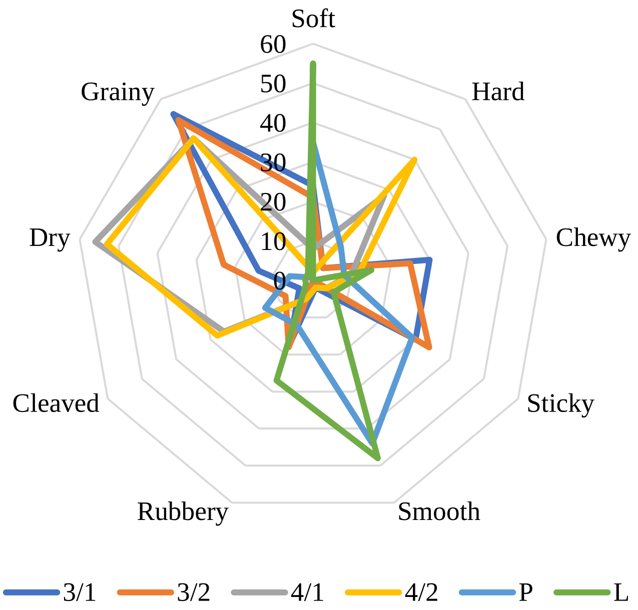


Figure 35. Results of the CATA-questions for the texture of the licorice samples (Table 11) in the second consumer test (n = 63, results as the amount of answers)

3.3.4 Other analyses

There were two different mechanical tests done with the samples, in order to get some knowledge of the physical properties of the samples, the compression and extension tests were conducted with a universal testing machine Instron. In addition, water activity was measured with two separate equipment (AquaLab & Novasina) to get more insight into the physicochemical properties of the licorice samples. Water activity measurements and compression tests could be conducted for all the self-made samples from the first batch as well as for the two commercial samples, but the extension test was only possible for the self-made samples from the first batch. The results of the analyses are presented below.

Water activity

The means for the water activity with two separate machines can be seen in Figure 36. AquaLab produced slightly higher values across the board for water activity. The water activity was higher in the self-made samples (0.671-0.681) compared to the commercial licorice (0.557-0.626), with the self-made sample with no anise and a smaller mass flow (1/1) having the highest a_w and the commercial sample from Lakritsfabriken having the lowest a_w.

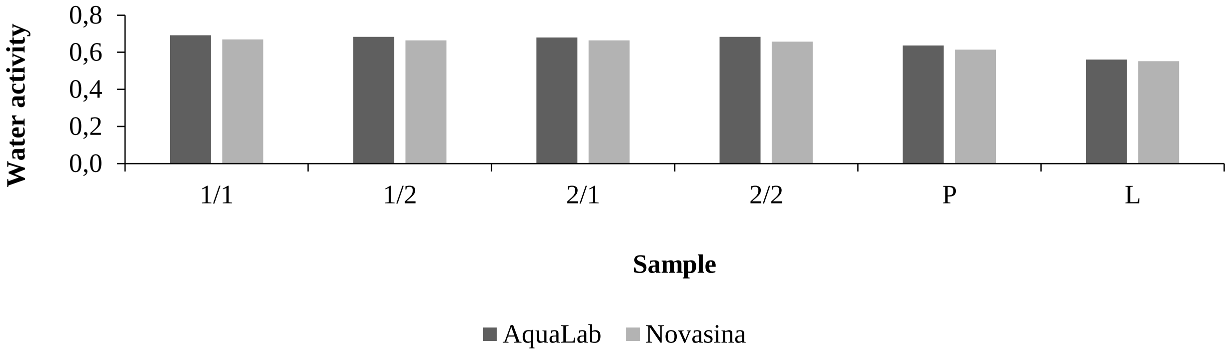


Figure 36. Water activities measured with AquaLab and Novasina

Extension

The extension test was conducted with Instron and 10 replicates were measured for better accuracy, but this measurement could only be done with the self-made samples. From the given values of the Instron-measurement, the tensile strength was calculated dividing the force at maximum load (N) by the displacement at the maximum load (mm) to gain the tensile strength (N/mm). All the samples got similar values for the tensile strength between 0.19-0.22 N/mm, the sample with anise and the smaller mass flow (2/1) getting the highest average value (Figure 37).

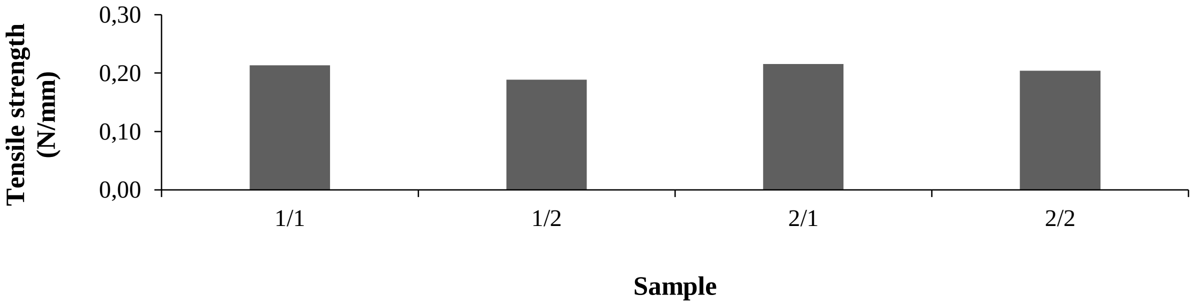


Figure 37. The tensile strength

Compression

The compression test was also conducted with Instron and 10 replicates were measured for better accuracy, and this measurement could be done with all the self-made samples as well as the commercial ones. From the given values of the Instron-measurement, the compressive strength was calculated dividing the primary force (N) by the compressive displacement (mm) to gain the compressive strength (N/mm). As seen in Figure 38, the samples got quite similar values, except for the commercial sample from Porvoon lakritsi, which got the highest value by far at 12.55 N/mm. The self-made samples got values between 3.74-7.48 N/mm, the sample with no anise and the smaller mass flow (1/1) having the highest value. Commercial sample from Lakritsfabriken got a value closest to sample 1/2 at 5.40 N/mm.

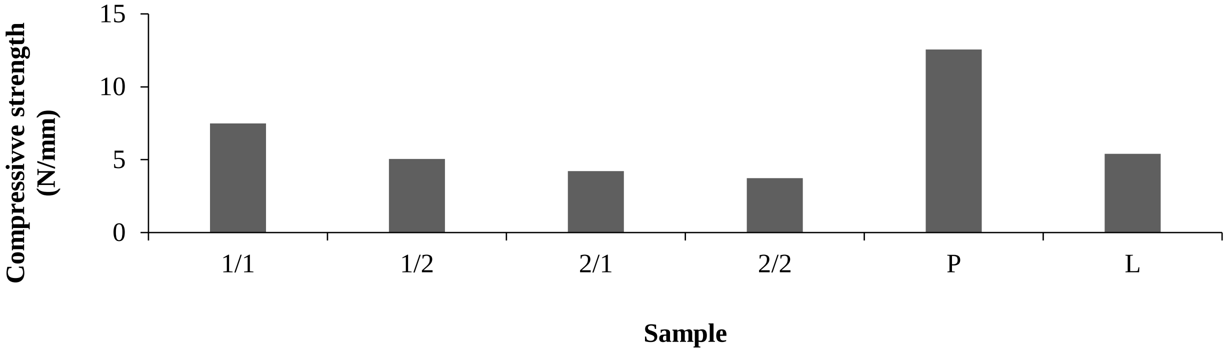


Figure 38. Compressive strength

The compression testing also revealed the maximum slopes of the samples, which can be seen in Figure 39. The commercial sample from Porvoon lakritsi had the smallest maximum slope with 38.5 and the self-made sample with anise and the smaller mass flow (2/1) had the highest maximum slope at 70.8.

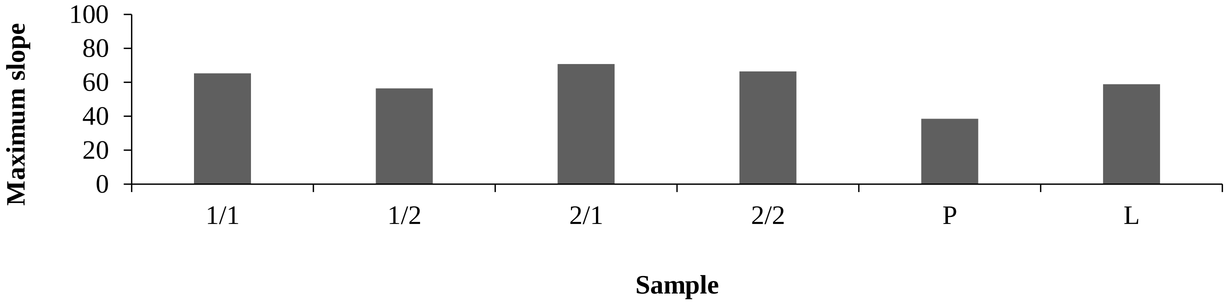


Figure 39. Maximum slope at compression test

3.4 Discussion

3.4.1 Sensory characteristics

Appearance

The first attribute described was the homogeneity of the appearance. The commercial samples seemed more homogenous in their appearance than the self-made samples, since the self-made samples only scored an average of 1.7 out of the possible 10, while the commercial samples ranked on average of seven. This difference between the self-made samples and the commercial samples is most likely reasoned with the fact, that the self-made samples had tiny quinoa particles stuck on the surface and this made them look grainy and not homogenous. The white spots have been an issue with this type of quinoa licorice in the previous research done at University of Helsinki. They might be caused by the quinoa flour not gelatinizing properly during the extrusion, but there is no certainty about, why the white spots appear on the surface, or what they even are exactly.

There have not been any spots on the surfaces of wheat or rice licorice made with the same extrusion, so this is a problem occurring just with the quinoa. This would indicate, that the spots are due to some property in the quinoa flour that does not exist in these other flours. The white spots could occur from poor gelatinization of the quinoa flour. However, even when the extrusion of quinoa licorice is successful, the white spots are still visible on the surface. It might be that some part or property of the quinoa flour does not react as wanted with the sugar and molasses of the licorice and therefore some of the flour particles are separated and rise to the surface. It has been studied, how sugars affect starch gelatinization in wheat by Beleia et al. (1996), but it is not clear if similar conclusions can be made with quinoa. At this point, it is impossible to say if the sugar-starch interactions are the reason for the white spots occurring in these licorice samples.

Another idea is that the protein content might also have something to do with the white spots. Quinoa has more protein (13-15%) compared to rice flour (7.5%), and it is close to the amount of protein in wheat flour (15.4%) (Abugoch et al., 2008). However, since the protein contents of wheat and quinoa are not that different, the problem might not have anything to do with proteins or their denaturation during the extrusion, since there have not been any white spots in the other samples and their flours have different protein features with each other.

The other attribute chosen to describe the appearance of the samples was shine. With shine, the results altered more widely than with the homogeneity, since there were three different groups that the samples were divided into, based on the Tukey's test. The commercial sample from Porvoon lakritsi was rated as the shiniest sample, which was no surprise, given that the licorice had a visible, glossy coating on the surface. This glossy coating differed significantly from the more matt appearance of the self-made samples as well as the other commercial sample by Lakritsfabriken, which was quite matt as well. In fact, the sample from Lakritsfabriken was rated as the least shiny, but from the ingredients, we can see that there were some glazing agents involved in the making of the licorice. The glazing agents were carnauba wax and coconut oil, which could be used to make the surface look matt on purpose. The shine of licorice is mostly affected by the degree of gelatinization according to Edwards (2000). Jackson (1990) stated as well that the more successful the gelatinization in licorice, the better the shine in the final product. This does not consider additional glazing agents like the ones in the sample from Lakritsfabriken to make it look matt on purpose.

One self-made sample differed from the others a little since it belonged to the same group with the commercial sample by Lakritsfabriken in the Tukey's test. This sample (1/1) had no added anise aroma and it was made with the smaller mass flow at 100 g/min. This could slightly indicate, that the absence of anise aroma in the ever so slightly thinner licorice would lead to a less shiny end-product. However, this difference to the other self-made samples was not significant. It could also mean that due to the smaller mass flow and the absence of the anise aroma, the gelatinization could have been a tiny bit poorer and this could have affected the perception of the shine (Jackson, 1990).

Odor

With this kind of samples, the odor is probably the most difficult category of sensory properties to describe. In general, the odor is one of the most difficult attributes to distinct and evaluate in sensory science, since people perceive odors differently (Berglund et al., 1975). The panel decided that they would break the samples a little to get the odor better out from the samples, but this was a hard category for a panel that had barely any experience. Therefore, there probably were not any significant differences between the samples in the overall intensity of the odor. In addition, with more training, the panel could have learned to recognize and evaluate odors and their overtones more distinctively. Then again, the odor and aroma are not the most important attributes for licorice acceptance (Anzmann, 2016). Even based on this test, we can see that flavor and texture have a bigger impact on the overall acceptance than the odor.

Concerning the odor intensity, the sample from Lakritsfabriken got the highest average, which might be due to all the additional ingredients of the licorice (glazing agents, additional sugars etc.) or the highest content of licorice extract in it (6%). From all the samples, the self-made one with no added anise aroma and bigger mass flow got the lowest score in odor intensity. This indicates that the bigger mass flow and thickness could hide the odor more than in the sample with a smaller mass flow, not to mention the samples with added anise aroma. With the sweet odor, there were more variations between the samples, since samples 1/2 and Porvoon lakritsi were rated significantly different from each other. As seen already with the overall intensity of the odor, also with the sweet odor the sample (1/2) with no anise aroma and the bigger mass flow got the lowest score. This emphasizes the small differences between the self-made samples, even though it was not significant. It also states that the glossy coating on the sample by Porvoon lakritsi could have had a sweeter odor and therefore made it stand out, especially from the self-made sample 1/2.

When comparing the results of the overall intensity and the sweet odor, we can see that the average score for the overall intensity of the odor was around six (scale from 0-10), while the average score for sweet odor was only four. This indicates that the samples smelled of something other than just sweet, which is understandable and normal. There are dozens of volatile compounds in the licorice extract alone, so it is no wonder that a complex food matrix like licorice has other scents than just sweet (Frattini et al., 1977). The overall intensity could be seen coming for example from the anise aroma and the cereal odors of the flour. There is around 1.5-5% of essential oils in anise oil alone, so there would definitely be other odors in licorice than just pure sweetness from sucrose (Ullah et al., 2014).

Texture

When the panel discussed describing the texture of the samples, they chose to focus on the homogeneity of the texture as well as the stickiness. From the results of the homogeneity of the texture, the same trend can be seen as with the results of the homogeneity of appearance. The self-made samples were described as less homogenous as the commercial samples. It can be that the self-made samples were, in fact, grainy in texture, but there is at least a chance that they remembered the grainy appearance and judged the texture to be grainy as well based on that. It is normal to “eat with your eyes” and it is hard to be objective about the texture when you have already seen the not homogenous appearance and swayed towards it affecting the texture as well (Delwiche, 2012). More training for the panel could have made a difference in the results if this was the reason for poor scores in the homogeneity of the texture for the self-made samples.

With the stickiness, the panel decided to evaluate this attribute based on the texture in the mouth, so it described more the sticking to the teeth and not the stickiness that might be found while holding the sample in hand. The commercial sample from Porvoon lakritsi got the lowest average as being the least one to be stuck to the teeth, while sample 1/2 was described as the stickiest of the samples. This attribute is also one of the more difficult ones to evaluate because it is rated in the mouth and other variables have an impact such as saliva and chewing time (Lawless and Heymann, 2010). On top of that, licorice is generally a chewy substance and it gets stuck to the teeth anyway, so it might be difficult to try and distinct the most sticking. Mouthfeel and stickiness to the teeth are sensory qualities that take time to master in evaluations (Guinard and Mazzucchelli, 1996). The samples were also quite different in thickness, which is a huge contributor to the sensation of texture. This made it challenging to compare the samples between each other and more training could have made it easier to evaluate this attribute.

Taste

The panel decided to choose four different attributes to describe the taste of the samples. The overall intensity of the taste was the first to be rated, followed by the descriptions of the sweet taste and the licorice taste (flavor). All of these three descriptions of the taste got similar results. The panel described the self-made samples to be significantly different from the commercial samples, but they could not identify differences between the self-made samples. One of the goals for the descriptive analysis was to see whether these types of differences in the manufacturing had any effect, and these results would indicate the parameters do not create significant sensory differences in the taste, which is a good result for further development.

With the rating of the licorice flavor, one would have expected some differences between the self-made samples that had anise aroma compared to the ones that did not, since anise aroma is most likely the reason for stating something to taste like licorice in the first place. However, it could be that the licorice extract dominated in the samples more, and by having the same concentration of that in all of the self-made samples, it might have diminished the differences between the added anise aromas. Licorice extract has dozens of volatile components that affect the taste perception and being sweeter and more dominant in the flavor than many other components, this is likely to have happened (Fenwick et al., 1990).

The fourth evaluated attribute of the taste was the intensity of the aftertaste. With the aftertaste, the results altered somewhat from the other taste descriptions. Here it seems that the samples with the smaller mass flows were described differently, compared to the ones that had bigger mass flows. The commercial samples also had a more intense aftertaste than the self-made samples. It seemed that there was something in the commercial samples, which made their aftertaste more intense than the self-made samples. Since there is no way of knowing how the commercial samples are manufactured, no certain conclusions cannot be drawn from that. However, the commercial samples were thicker and bigger than the self-made samples, so the results for aftertaste might just be due to that. Lastly to the differences between the self-made samples; the samples with the bigger mass flows were rated with a more intense aftertaste. It was interesting to see that with the aftertaste the self-made samples with bigger mass flows were seen more intense, but when the odor was rated the same samples had the least intense overall and sweet odor. These were the only inklings to the hypothesis that the process parameters could affect the sensory properties, but since they were seen only in a couple of attributes out of ten, this needs further research before any of this can be conclusive in any way.

3.4.2 Panel performance in the descriptive analysis

Descriptive sensory analyses aim to profile products on all of their perceived sensory characteristics (Murray et al., 2001). All descriptive methods require a panel with some degree of training or orientation. These types of studies can only be conducted with a panel, which has undergone at least one training session since the descriptive attributes and scales are decided on in the training session (Lawless and Heymann, 2010). Typically, there is more than one training session with a generic descriptive analysis, but this study only had one training session due to a tight schedule. This could have affected the results in multiple ways. The problem with just one training session is, that the panelists do not perhaps have enough time to comprehend and internalize all the descriptive attributes or feel comfortable with the evaluation and its techniques (Moskowitz, 2012). Also, one training session might not be enough time for the whole panel to get on the same page, meaning that the variation between the answers can be more distinct. This can cause bigger errors and bias results if the panelists do not understand the terms and scaling similarly.

According to Murray et al. (2001), the most important thing for a successful sensory evaluation project is a panel that is committed and motivated to go through with the evaluations. This might not have been completely achieved with the panel in this study. The panel consisted of 19 food science students and participation was part of their course work, which means that the commitment was there, since they had to be a part of a panel to pass the course. The mandatory judging could, however, affect the panelists in a way that might have caused problems in the motivation of doing the evaluation properly. In theory, someone could have just done the evaluation without really focusing on the questions or descriptions, of course, this being the case in all sensory evaluations, due to private evaluations (Tuorila and Appelbye, 2008). However, the course was not mandatory so we can assume that on some level the students who participated at the panel are interested in sensory science and took their education seriously enough to perform at a good level.

Another sign of good panel performance is the fact that the results can be trusted and analyzed properly (Lawless and Heymann, 2010). In this descriptive analysis, the three-way analysis of variance was done to see how well the panel performed. From the results of the multiple comparisons, we can see that the panel noticed significant differences between the samples. This was expected and indicates good panel performance since the samples were different in their ingredients and process parameters. We can also see that there were no significant differences between the results of the two separate sessions. This is a good indicator of the reliability of the panel (Tuorila and Appelbye, 2008). It means that the panelists evaluated the samples similarly in both sessions, with the same range of values.

From the results concerning the panelists, we saw how there were significant differences in them. This could be expected as well, and it means that single panelists evaluated the samples with different parts of the scales, some people using the lower end and some the higher end of the scale for all of their descriptions. This is not preferable, but in real evaluation settings, it will happen, although with more extensive training it can be decreased significantly (Labbe et al., 2004). The real interests, however, were the interactions of the three combination categories in the panel performance. Firstly, with the interaction between the sample and the session, we can see that the panelists stayed in the same magnitude of scale during the two sessions, meaning that they evaluated the same sample around the same value in both sessions. This is a good sign of reliability since it would be a sign of poor judgment to evaluate the same sample with two totally different values (Rossi, 2001).

When focused on the interaction with the sample and the panelist, it showcased how different panelists rated the samples with different values and since the samples were different from each other, this was a good and reliable result as well. The samples were different based on their ingredients and process parameters, so if the panelists would have rated them similar and had not found any differences, the panel would have performed very poorly (Rossi, 2001). The better the differentiation between the samples, the more reliable and repeatable the study is. This interaction also showed that the samples were different enough so that differences could be perceived by the panel to some extent, but as seen from the results of the descriptive analysis, they were not significant in a statistical manner. The last interaction was the one between the session and the panelist, which is the most important one for seeing whether or not the panel worked well. With this interaction, there were no significant differences between the attributes, which is a good thing. It means that the panelists rated the same attribute with the same magnitude of scale in both sessions, which is a great indicator of good repeatability in the panel (Naes, 1990). In conclusion, the panel worked well, and the results are good and reliable.

3.4.3 Consumer opinions

In both consumer tests, background information was collected from both panels (87 people in the first panel and 63 people in the second panel). The consumption of sweets and licorice between the two panels were similar to each other. In both panels, the majority of the panelists (>93%) ate sweets weekly or more than a couple of times a month. When it came to licorice, in both panels more than 60 % ate licorice at least once a month, but 30 % also ate licorice only once a year or less. This confirmed the assumption, that most of the panelists were frequent users of sweets and licorice, which made them an adequate panel. The panelists also had to describe the type of licorice they usually eat and again the two panels were somewhat alike, with both panels eating mostly plain and filled licorice, followed by salmiac and coated licorice types.

Pleasantness of the licorice

The two consumer tests are difficult to compare since the self-made samples were different in them, but some comparisons can still be made. The scales for the consumer tests were hedonic nine-point scales with verbally anchored ends. This means that all scores were between one and nine, nine being the highest and meaning that the sample was very pleasant concerning the attribute in question.

<u>Appearance</u>

When the samples were evaluated in the pleasantness of appearance, differences and similarities between the two tests can be seen. In the first consumer test, the commercial sample by Porvoon lakritsi seemed most pleasant according to the panel and the same sample was found the most pleasing in the second test as well. Also, with the other commercial sample from Lakritsfabriken, the results were quite similar in the pleasantness of the appearance, since it was rated as the second-best in both consumer tests. In both consumer tests, the quinoa licorice samples were rated about three points lower than the commercial licorice samples, which on a nine-point scale is quite a big difference as a third. The main reason for the poor ratings in the appearance might be due to the grainy surface of the quinoa samples. The rice licorice samples in the second consumer tests also got fairly low scores compared to the commercial samples, however, they were rated more pleasant in their appearance than the quinoa samples. The low scores of the self-made samples might be due to the thin and stringy appearance them, which obviously was not seen as pleasant as the thicker and more homogenous appearance of the commercial samples.

Odor

With the pleasantness of the odor, the sample by Porvoon lakritsi got the highest scores again in both consumer tests. This could be well connected with the descriptive analysis results. There, sample by Porvoon lakritsi was rated as the sample with the sweetest odor and this was then seen as the most pleasant by both panels. This type of acceptance for sweet odors and flavors is typical and fairly expected with confectionery products like licorice (Yeomans et al., 2006). For all the quinoa samples, both panels rated the pleasantness of the odor quite similarly on average around 5, and in the second consumer test, they were seen similar to the rice samples as well. With the commercial sample from Lakritsfabriken, the evaluations varied the most between the two consumer tests. In the first consumer test, it was rated as the second-best (6.0), while in the second test it got the lowest value (4.9) being the least pleasant according to odor. This difference might be due to anything from quickly evaporating odor to difficulty in getting the odor sensed from the sample, so nothing certain cannot be said about it. According to Berglund et al., (1975) odor is one of the most difficult attributes to distinct and evaluate in sensory science since people perceive odors differently. Since the consumers were not expert panelists their conception and technique during the odor evaluation especially can have a great effect on the results.

Taste

When it comes to the pleasantness of taste, the commercial sample by Lakritsfabriken got the highest scores in both tests. It seemed, that even if it scored lower than the other commercial sample both in the pleasantness of appearance and odor, this sample by Lakritsfabriken tasted better. The same change can be seen between the quinoa and rice samples in the second consumer test. With both previous attributes, the rice samples got higher scores than the quinoa samples, but with the pleasantness of the taste, quinoa samples got better scores than the rice samples for the first time. The quinoa samples seemed more pleasing in their taste in both tests, since they scored an average around 4.2, while the rice samples only scored 3.3. This indicated that the rice licorice looked and smelled better but tasted worse, however, the differences were not huge and not significant.

Texture

The fourth category in the evaluation was the pleasantness of the texture, and it was evaluated as the texture in the mouth while chewing the samples. With this attribute, the commercial samples got the highest scores in both tests, sample by Porvoon lakritsi being the panel favorite. It is interesting, how the sample by Lakritsfabriken seemed to taste better, but all the other attributes were found more pleasing with the sample by Porvoon lakritsi. This means that the taste was not the only main reason for the total acceptance, the texture, appearance, and odor had an effect also. The self-made samples were rated with lower scores compared to the commercial samples, and the biggest possible reason for that might be the significant difference in the thickness of the samples. There is only one nozzle (Ø 5 mm) fitted for licorice extrusion here at the university and since expanding is not expected in licorice, the thickness of the samples was left quite small.

Overall pleasantness

In the rating of overall pleasantness for the samples, the commercial sample by Lakritsfabriken ranked the highest in both consumer tests. Since it was rated higher than the sample by Porvoon lakritsi in only one attribute separately, the taste, it is reasonable to state that the taste of the licorice contributes the most to the overall liking of the sample. With the self-made samples, all quinoa samples ranked higher than the rice samples in overall pleasantness. This is a positive result for the overall quinoa licorice research. However, the poor extrusion results of the rice samples most likely affected the pleasantness ratings. Since the rice samples had different ratios of ingredients, the taste and texture were not as wanted. It would be interesting to see in a new study, what the consumers thought about the rice samples if the samples would be closer to the taste and texture of the quinoa samples.

3.4.4 Evaluation of the texture

The texture of the samples was evaluated with a check-all-that-apply section in the sensory analyses as well as with instrumental testing that included determination of water activity, and extension and compression tests.

Check-all-that-apply

The last part of the consumer tests was a quick check-all-that-apply section focusing on the texture of the samples. The samples from Porvoon lakritsi and Lakritsfabriken were quite similar in both consumer tests, as they were both described as smooth and soft. In industrial, continuous processing it is possible to increase the boiling temperature so that gelatinization can occur throughout the licorice cooking process (Hartel et al., 2017). While this allows the licorice to be more homogenous, it also tends to over-gelatinize the starch leaving the final product to be rubberier in texture. This would explain why the commercial samples were rated rubberier in both consumer tests in the CATA section. In the first consumer test, all the quinoa samples got similar scores and were described as grainy and dry. In the second consumer test, the quinoa samples were described as grainy, chewy and sticky again. The rice samples were of course only evaluated in the second consumer test and they were seen as dry, grainy, cleaved and hard.

Water activity

The texture was evaluated with the CATA section from the sensory perspective, both other analyses were also conducted to see the properties of the licorice texture. The water activity tells the degree of free water in the matrix and it describes the water that is available for reaction and microbial growth in the food (Katz and Labuza, 1981). The lower the a_w value, the more water is already attached to the different compounds in the food item and this attached water makes the item more stable and unavailable for microbes and spoilage. Water activity can have an effect on food texture (Sancho-Madriz, 2003). The reactions that are caused by the “free” water can alter the humidity and stability of the food. It can be controlled by adding humectants to food items so that the possible changes by the reacting water would not affect the texture and shorten the shelf-life of the products.

Water activity was higher in the self-made samples (0.671-0.681) compared to the commercial licorice (0.557-0.626). These results fair nicely compared to other research about licorice, studies like Rytkönen (2017) and Kallio (2006) stated the water activity of fresh licorice around 0.677-0.718.

They also determined the water activity four months after the manufacturing and got the results around 0.508-0.642. Since the values of this study were measured ten weeks after the manufacturing and were 0.671-0.681 for the quinoa samples, the results are reliable, and they scale well compared to water activities from previous studies.

Mechanical testing

The texture, or structure as referred to in the physical form, was also studied with extension and compression tests. Tensile stress is the stress caused by an applied load that tends to elongate the material by pulling it (Shama and Sherman, 1973). Tensile strength is the limit where the tensile stress leads to failure in a material. In the extension test here, all the samples got similar values for tensile strength at 0.189-0.216 N/mm, the sample 2/1 getting the highest value. Compared to research done by Rytkönen (2017) these values for extension were higher than seen in that study for the fresh samples (0.027-0.048 N/mm), but somewhat lower than the values for the samples after four months of storage (0.123-0.373 N/mm). In that research, the tensile strength was measured right after the manufacturing and again after four months, while in this study the samples were measured only once, 10 weeks after manufacturing. The results from this study sit nicely in between the results from her two measurements since mine were measured time-wise in between those, although closer to the four-month timepoint.

Compressive stress, on the other hand, is the stress caused by an applied load that tries to squeeze the material, in its simplicity, it is the compression action of two opposite pushing forces (Shama and Sherman, 1973). Compressive strength is the limit where the compressive stress leads to failure in a material. With the compressive strength measurement in this study, the commercial sample from Porvoon lakritsi got the highest value by far at 12.55 N/mm. The self-made samples got values between 3.74-7.48 N/mm, the sample with no anise and the smaller mass flow (1/1) having the highest value. Commercial sample from Lakritsfabriken got a value closest to sample 1/2 at 5.40 N/mm.

Similarities between these compression results and the texture profiles of the CATA evaluation from the consumer tests can be found. In both consumer tests, the sample from Porvoon lakritsi was seen more cleaved and harder than the other commercial sample by Lakritsfabriken, which would explain the better resistance of the force as well. Since the sample from Lakritsfabriken was rated as soft and chewier (referring to elasticity) it supports the fact that it got a smaller compressive strength than the sample from Porvoon lakritsi. This could have been reinforced with the extension test, but unfortunately, the commercial samples were too short for that test.

The self-made samples are a bit harder to compare since they were different in the two consumer tests. However, most of the self-made samples were rated as dry and hard in both consumer tests, which could correlate to their compressive strength values, but since they were significantly thinner than the commercial samples by Porvoon lakritsi they did not result in such high strength values that could be expected by the sensory ratings. There have not been significant compression tests done for licorice before, so there were no comparable references for that. However, according to Shama and Sherman (1973), compressive strength is typically bigger than the tensile strength of a food item, and in that account, these samples were spot on.

3.4.5 Observations from the extrusion process

Issues with the process

There were issues with the extrusion processes in this research. On both extrusion times the licorice was too runny in the beginning, and therefore some of the process parameters (mass flows and feeding rates) had to be adjusted during the process. The feeding rates of the flour were increased so that the licorice samples would not be as runny, and therefore the mass flows increased as well. With the first batch, the mass flow was one of the changing variables between the samples. The original mass flows 80 and 120 g/min had to be increased to 100 and 130 g/min. With the first batch, this runny licorice was not expected at this scale, since the same mass flows (80 and 120 g/min) had been used in multiple previous research and the pre-tests of this study (Kallio (2006); Anzmann (2016); Rytkönen (2017)). However, the extrusion results have not been consistent in the previous studies either, so the fact that there were some problems was not a total surprise.

One reason for the runny licorice could have been, possible miscalculations in the recipe or the feeding rates, probably more in the latter. Although the feeding rates were tested before the extrusion process, and if there was an error, it might not have been noticeable before the actual extrusion. Another possible explanation for the runniness of the samples could be the low water content of the quinoa flour. Compared to other literature sources (Ogungbenle (2003); Rytkönen (2017)), this quinoa flour batch was the driest and this could have affected the properties of the solid ingredients, for example, their water absorption and gelatinization ability. However, the water contents of the samples were calculated, and the moisture content of flour was acknowledged so this could have no effect on the runniness. These small differences or errors compared to the previous, similar licorice studies could explain the runny samples with batch number one.

With the second batch, the quinoa licorice succeeded well. This time it seemed that the slightly higher mass flow of 100 g/min worked better than the originally planned 80 g/min, and the quinoa samples turned out just fine from the start. The rice licorice was then a whole other case. This type of rice licorice had never been made with this extruder, and there was not enough time to search for a proper recipe for the licorice. A decision was made to use the same recipe base that the quinoa licorice had for the rice licorice as well. In hindsight, it should have been better to try out the rice licorice recipe first, since it clearly did not work.

While the runny quinoa licorice in the first batch already looked like licorice even while being too soft, the runny rice licorice was just a hot liquid mixture when it came out from the extruder the first time. There were time-limitations to get the rice licorice out and working for the sensory evaluations, so the changes for the process parameters had to be done during the extrusion process. The addition of the flour mixture was done by the cost of the liquid mixture and in the end, the licorice had the flour-liquid ratios completely opposite from the quinoa licorice. The flour feed had almost doubled, while the amount of liquid had decreased to a quarter from the original values. This led to a very different type of product with the rice licorice compared to the quinoa licorice, the rice licorice being super hard, dry and floury. For further studies, a proper recipe and more detailed testing with the extruder would be needed if any type of rice licorice were to be made again.

Problems with starch gelatinization

Another reason for all the runny samples could be poor gelatinization of the flours during the extrusion. According to Heldman and Hartel (2012) the preferred temperature for licorice making is 150 °C when in this extrusion, the highest temperature was 145 °C. With higher process temperatures the gelatinization might have been more complete. One of the most important properties of starch is its ability to absorb water and gelatinize when heated. Gelatinization happens in two phases. In the first phase the granules swell rapidly, and in the second phase (temperature over 90 °C) the granules swell even more and lose their shape, the structure of the starch granule breaks, and it gelatinizes.

According to Huang and Rooney (2001), the degree of gelatinization of the starch depends on the used temperature, water content and other elements of the cooking (pH and the number of solid particles). The temperatures in this study were the same as they have been in several previous studies with quinoa licorice, so it should not have affected the extrusion results. The water content (24%) was also close to those of several previous studies (23.5-24%), so there should not be any differences caused by that either. However, this time the water contents of the liquid ingredients were not calculated, but the specification values from the manufacturers were trusted. This might have made a slight change in the dough and therefore in the gelatinization properties. What comes to the other elements of the cooking such as pH or the number of solid particles, there is no reason to doubt they would differ from the values in previous research on similar quinoa licorice samples. None of these elements though have been measured in this or the previous studies.

Amylopectin is responsible for the swelling of starch granules since it has higher water absorption abilities compared to amylose. Quinoa contains a lot of amylopectins and has great water absorption properties (147%), so the water absorption properties of quinoa are most likely not the cause for the differences in gelatinization (Ogungbenle, 2003). Edwards (2000) and Rytkönen (2017) both stated how the gelatinization degree of the starch could affect the structure substantially and this could explain the big problems especially with the rice licorice. In addition to the properties of amylopectin, the properties and content of amylose are also important in starch gelatinization (Edwards, 2000). If the level of amylose is too high, then instead of forming a gel the amylose will liquefy the starch and produce a sticky goo, like happened here with the rice licorice. Overall, more research and more repeated studies should be made with this type of extrusion and with all types of licorice, to get a good and stable product out every time.

Differences in water contents

There were differences between the water contents of the solid ingredients in the two batches. The water contents of the solid ingredients were measured by drying them in heat cabinets and calculating the weight loss into the percentage of water. The first batch of ingredients was dried in a vacuum heat cabinet, while the second batch of ingredients was dried in a normal heating cabinet. The method used with the vacuum heat cabin is not standardized, but a similar drying method was used by Rytkönen (2017) in a previous study with similar samples. The second batch was dried with a tested method accepted by AACC (1981). The differences in the drying methods may explain some of the variations between the water contents of the solid ingredients.

Concerning the quinoa flour, the natural explanation for the slight difference between the two batches in their water contents is the different batches of flours, since every grain and flour batch is different. The normal differences are due to cultivation conditions, harvest and milling processes. Ogungbenle (2003) stated that the water content in quinoa flour is 11.2% and there has been previous research on similar quinoa licorice where the water content of quinoa flour has been 10.2% and 12.3% (Rytkönen, 2017). The batches of quinoa in this study were even drier than the ones found in literature, with water contents at 9.5% and 9.9%. This might be due to differences in the method of calculating the water contents, drying the flours or the differences in the quality and origin of the quinoa flour. With the rice flour, there was only one batch used in this study, so there was no comparison between two batches. However, based on literature, the water content of rice flour is preferred under 20%, so our rice flour was at least within the frames of that at 10.54% (Marshall and Wadsworth, 1994).

The difference in water contents between the two batches of licorice extract is the most substantial from all the solid ingredients. These licorice extracts had water contents at 7.3% (batch 1) and 9.3% (batch 2). Besides the difference in the drying methods, these licorice extracts were probably the most different from the start. The licorice extract powder used with the first licorice batch was older and the package had been opened for months. The powder was kept in an airtight container, so we do not know how much contact it had with the surrounding environment, but it seemed to have dried out a bit. The powder used with the second batch of licorice was a mixture of two powders, the same old one used with the first batch and a brand new, fresh one straight from the manufacturer. We do not know how long and how the manufacturer stores the powder, so again we do not know how much contact the powder had with the environment. We cannot say for sure what caused the bigger (2%) difference in the water contents of the licorice extracts. We can, however, state that the fresh licorice extract contained more water than the old one. According to Fenwick et al. (1990), a fresh licorice extract can have a moisture content around 11-13%. Similar licorice extract used at the University of Helsinki previously has had a smaller water content than Fenwick et al. (1990) stated, since both Rytkönen (2017) and Anzmann (2016) found their licorice extract to have the water content at 10.12%. It would seem, that the new batch of licorice extract had a higher moisture content (10-12%), and the old one had it similar to batch one (~7%) so that the average water content for the mixed licorice extract ended up being there around 9%.

4 Conclusion

The goal of this study was to develop gluten-free quinoa licorice with a twin-screw extruder and determine its sensory and physical properties. The quinoa licorice making was achieved as well as the additional, rice licorice making, although that resulted in a poor product. The recipe for rice licorice was not optimal and it needs further development before reusing. Sensory properties of all the self-made licorice samples, together with two commercial samples, were conducted with a generic descriptive analysis and two separate consumer tests. Other analyses were also done to determine some physical properties like extension and compression.

The hypothesis of the research was that the differences in the process parameters (aroma, mass flow) would lead to significant differences in the sensory and physical properties of the self-made samples, however, they did not. The sensory profile of quinoa licorice samples showed that they were evaluated as significantly different from the two commercial samples, but not from each other. The self-made samples were seen as dry and grainy, whilst the commercial samples were rated as softer and chewier.

The consumer tests showed that the panels preferred the commercial samples over the self-made licorice samples. These results showed that the pleasantness of the taste had the biggest effect on the overall pleasantness of the samples. The commercial sample by Lakritsfabriken was rated more pleasant in the overall pleasantness, despite scoring lower values than the sample from Porvoon lakritsi in all separate categories expect the pleasantness of taste. The self-made samples also scored lower than the commercial samples in taste in both consumer tests, which led to poorer results in overall pleasantness. In the second consumer test, the quinoa licorice samples also got better overall reviews since they scored higher in their taste than the self-made rice licorice samples.

The results from the mechanical tests showed that the differences between the self-made and commercial samples were good in comparison to literary values from previous research. Similarities between the compression results and the texture profiles of the CATA evaluation from the consumer tests were found. The sample from Lakritsfabriken was rated softer and chewier than the sample by Porvoon lakritsi. This correlated with the compressive strength of the sample by Porvoon lakritsi being higher than that of Lakritsfabriken’s sample.

For further studies with quinoa licorice, the focus could evolve around the development of the texture and flavor, in order to get a product that could compete with the traditional wheat licorice. On the other hand, this type of gluten-free product would be mainly marketed for people who cannot use wheat in their diet. Therefore, a better product that fairs better in comparison with commercial gluten-free licorice should be the primary focus.

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APPENDICES

Appendix 1. The water contents of the solid ingredients

Batch one

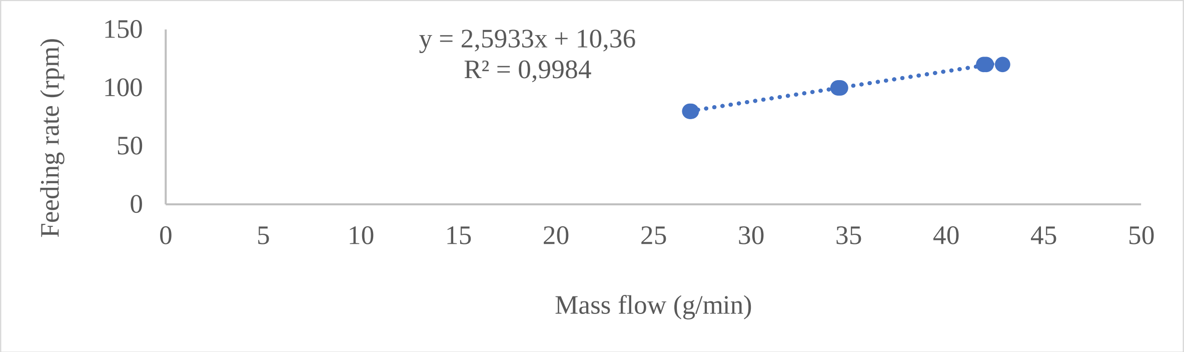
Sample	Empty cup (g)	Filled cup before (g)	Filled cup after (g)	Flour before (g)	Flour after (g)	Weight loss (g)	Water content (g)	Water content (%)
Quinoa 1	55,2176	56,3552	56,2469	1,1376	1,0293	0,1083	0,0952	9,5200
Quinoa 2	50,5334	51,6572	51,5500	1,1238	1,0166	0,1072	0,0954	9,5391
Quinoa 3	52,9420	53,9849	53,8855	1,0429	0,9435	0,0994	0,0953	9,5311
Licorice extract1	51,9528	52,9600	52,8865	1,0072	0,9337	0,0735	0,0730	7,2975
Licorice extract 2	51,4076	52,4158	52,3422	1,0082	0,9346	0,0736	0,0730	7,3001
Licorice extract 3	51,9512	52,9909	52,9148	1,0397	0,9636	0,0761	0,0732	7,3194

Batch two

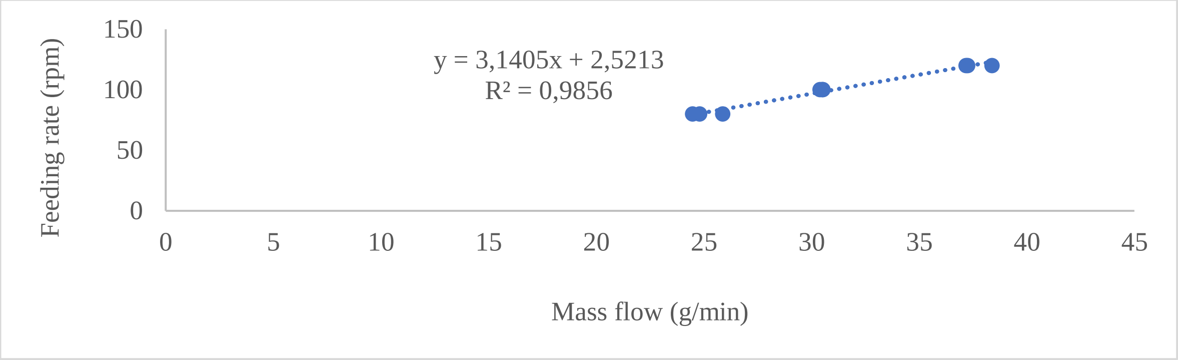
Sample	Empty cup (g)	Filled cup before (g)	Filled cup after (g)	Flour before (g)	Flour after (g)	Weight loss (g)	Water content (g)	Water content (%)
Quinoa 1	50,9554	53,0724	52,8639	2,1170	1,9085	0,2085	0,0985	9,8488
Quinoa 2	49,2385	51,2119	51,0184	1,9734	1,7799	0,1935	0,0981	9,8054
Quinoa 3	51,5217	53,8441	53,6104	2,3224	2,0887	0,2337	0,1006	10,0629
Rice 1	52,6443	54,8199	54,5961	2,1756	1,9518	0,2238	0,1029	10,2868
Rice 2	52,2465	54,3983	54,1723	2,1518	1,9258	0,2260	0,1050	10,5028
Rice 3	50,8358	53,0028	52,7682	2,1670	1,9324	0,2346	0,1083	10,8260
Licorice extract 1	51,1903	53,3812	53,1863	2,1909	1,996	0,1949	0,0890	8,8959
Licorice extract 2	51,2231	53,4391	53,2261	2,2160	2,003	0,2130	0,0961	9,6119
Licorice extract 3	49,8506	51,8891	51,702	2,0385	1,8514	0,1871	0,0918	9,1783
Licorice extract 4	51,0850	53,3318	53,1205	2,2468	2,0355	0,2113	0,0940	9,4045
Licorice extract 5	51,1516	53,3070	53,1043	2,1554	1,9527	0,2027	0,0940	9,4043
Licorice extract 6	50,8207	52,9781	52,7756	2,1574	1,9549	0,2025	0,0939	9,3863

Appendix 2. Feeding rate equations for the second batch

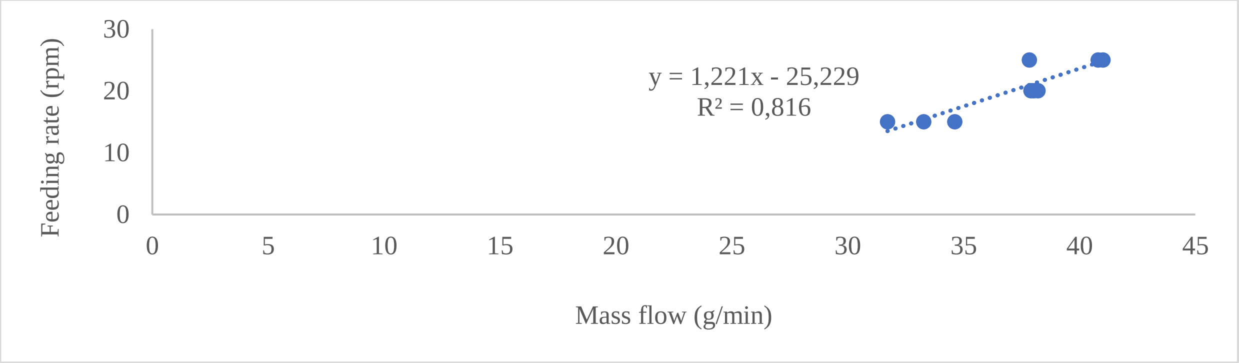
Mass flow of the quinoa flour in second batch as the function of feeding rate



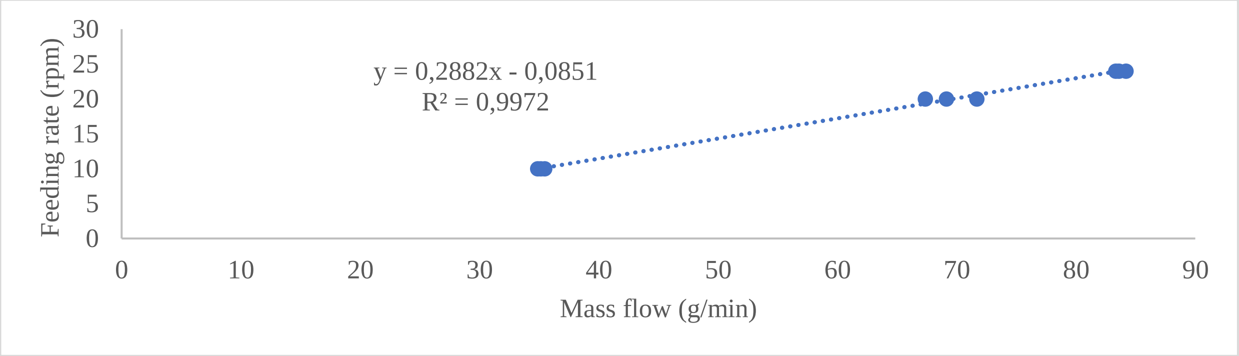
Mass flow of the rice flour in second batch as the function of feeding rate



Mass flow of the liquid sugar in second batch as the function of feeding rate



Mass flow of the coconut syrup in second batch as the function of feeding rate



Appendix 3a. Descriptive analysis questions

University of Helsinki, Department of Food and Nutrition, Sensory Science

FOOD-116 Advanced Sensory Science (2018), Exercise 1: Generic Descriptive Analysis

Panel 2: Licorice

Attributes and anchors for end points and reference (R)

Note: Numbers, tick marks, and location of the reference (R) exist on these training scales only (not on computer screen).

Appearance

1. Homogeneity of appearance

Grainy **R** **Smooth**

0 10

2. Shine

Matt = R **Shiny**

0 10

Odor (orthonasal)

3. Overall intensity of odor

No odor **Strong odor**

0 10

4. Sweet odor

No odor **Strong odor**

0 10

Please turn over

Appendix 3b. Descriptive analysis questions

Structure (in the mouth)

5. Homogeneity of structure

Grainy **Smooth**

0 10

6. Stickiness

Not at all sticky **Very sticky**

0 10

Taste

7. Overall intensity of taste

Mild **Strong**

0 10

8. Sweetness

Mild **Strong**

0 10

9. Licorice taste

Mild **Strong**

0 10

10. Intensity of aftertaste

Mild **Strong**

0 10

Appendix 4a. Consent form of the first consumer test

University of Helsinki, Department of Food and Nutrition, Sensory Science Laboratory
P.O. Box 66 (Agnes Sjöbergin katu 2, EE Building, 2nd Floor), 00014 University of Helsinki

Informed consent to participate in a sensory study

General principles of sensory research
In sensory research, information on characteristics of foods and human responses to foods are collected. This information is collected through the senses: by looking, touching, smelling, and tasting of food samples or their ingredients. At the beginning of a sensory test, the purpose of the study is explained to the participants to the extent that is possible without leading the respondent. After the test, a briefing session where the objectives and results are reported in more detail, can be arranged. If necessary, the participants can also request information by email. All samples evaluated by tasting in the sensory laboratory meet the requirements of food legislation. Personal information of the participants is handled confidentially and separately from the actual research data.

General principles of the consent
With this consent, the participant agrees to follow the instructions given for the study. A person has right to refuse to participate in the study. The participants have also right to cancel their participation anytime during the test. No reasons need to be given for the cancellation and it will not affect the treatment of the person who cancelled her/his participation. The data provided by the person who cancelled her/his participation will not be used in the study.

Information on the study that the respondent agrees to participate by signing this consent

Name of the study:	Consumer study on gluten-free licorice
When the tests are conducted:	**February 7th and 8th, 2018**
Quality and number the samples:	**Six licorice samples**
Swallowing of the samples:	The samples may be swallowed or spit out
Number and duration of test(s):	**One session, about 15 min**

Ingredients that can potentially evoke allergic reactions or intolerance: **Licorice extract. The samples are made of gluten-free ingredients, but in a space where gluten is handled.**

Researcher in charge of the study: University Lecturer **Antti Knaapila.**
Contact information of the researcher in charge: Ph: +358 50 316 5908, **antti.knaapila@helsinki.fi**

Consent
I have been adequately informed about this study. I have considered the samples and confirm that they do not contain ingredients that could cause allergic reactions or intolerance to me. I consent voluntarily to be a participant in this study and allow my responses to be used for research.

Helsinki ______. ______.201____

____ ____________________________________

Signature* Name in block letters*

E-mail address: ____

Phone number: ____

Appendix 4b. Consent form of the second consumer test

Informed consent to participate in sensory research

General principles of sensory research

In sensory research, information on characteristics of foods and human responses to foods are collected. This information is collected through the senses: by looking, touching, smelling, and tasting of food samples or their ingredients. At the beginning of a sensory test, the purpose of the research is explained to the participants to the extent that is possible without leading the respondent. After the test, a briefing session where the objectives and results are reported in more detail, can be arranged. If necessary, the participants can also request information on the study by email. All foods and ingredients assessed in the sensory laboratory meet the requirements of food legislation. Personal information of the participants are handled confidentially and separately from the actual research data.

General principles of the consent

With this consent, the participant agrees to follow the instructions given for the study. A person has right to refuse to participate in the study. The participants have also right to cancel their participation anytime during the test. No reasons need to be given for the cancellation and it will not affect the treatment of the person who cancelled her/his participation. The data provided by the person who cancelled her/his participation will not be used in the data analyses.

Information on the study that the respondent agrees to participate by signing this consent

Name of the study:	**Consumer study on gluten-free licorice**
When the tests are conducted**:**	**April 25th and 26th, 2018**
Quality and number the samples:	**Six licorice samples**
Swallowing of the samples:	**The samples may be swallowed or spit out**
Number and duration of test(s):	**One session, about 15 min**

Ingredients that can potentially evoke allergic reactions or intolerance: **Licorice extract. The samples are made of gluten-free ingredients, but in a space where gluten is handled.**

Researcher in charge of the study: University Lecturer **Antti Knaapila**

Contact information of the researcher in charge: Ph: +358 50 316 5908, **antti.knaapila@helsinki.fi**

Consent

I have received adequate information about this study. I have considered the samples and confirm that they do not contain ingredients that could cause allergic reactions or intolerance to me. I consent voluntarily to be a participant in this study and my responses may be used for scientific purposes.

Helsinki ______. ______.2018

____

Signature* **Name in block letters***

Email address: ____

Phone number: ____

Appendix 5. Background information form in both consumer tests

Tervetuloa lakritsin kuluttajatutkimukseen! - - *Welcome to the consumer study of liquorice!*

Vastaa ensin lomakkeen kysymyksiin ja tee sitten lakritsinäytteiden arviointi tietokoneella. *Please first complete this questionnaire and then evaluate the liquorice samples with the computer.*

1. Mikä on sukupuolesi? / *What is your gender?*

___ Nainen / *Female* ___ Muu / *Other*

___ Mies / *Male* ___ En halua vastata / *Prefer not to answer*

2. Minkä ikäinen olet? / *How old are you?*

__________-vuotias / *years old* ___ En halua vastata / *Prefer not to answer*

3. Kuinka usein syöt karkkia tai suklaata <u>keskimäärin</u>? / *How often do you eat candy or chocolate <u>on average</u>?*

- ☐ Kerran viikossa tai useammin / *Once per week or more frequently*
- ☐ Muutaman kerran kuukaudessa / *A couple of times per month*
- ☐ Kerran kuukaudessa / *Once per month*
- ☐ Muutaman kerran vuodessa tai harvemmin / *A couple of times per year or less frequently*
- ☐ En osaa sanoa / *Cannot say*

4. Kuinka usein syöt lakritsia <u>keskimäärin</u>? / *How often do you eat liquorice <u>on average</u>?*

- ☐ Kerran viikossa tai useammin / *Once per week or more frequently*
- ☐ Muutaman kerran kuukaudessa / *A couple of times per month*
- ☐ Kerran kuukaudessa / *Once per month*
- ☐ Muutaman kerran vuodessa tai harvemmin / *A couple of times per year or less frequently*
- ☐ En osaa sanoa / *Cannot say*

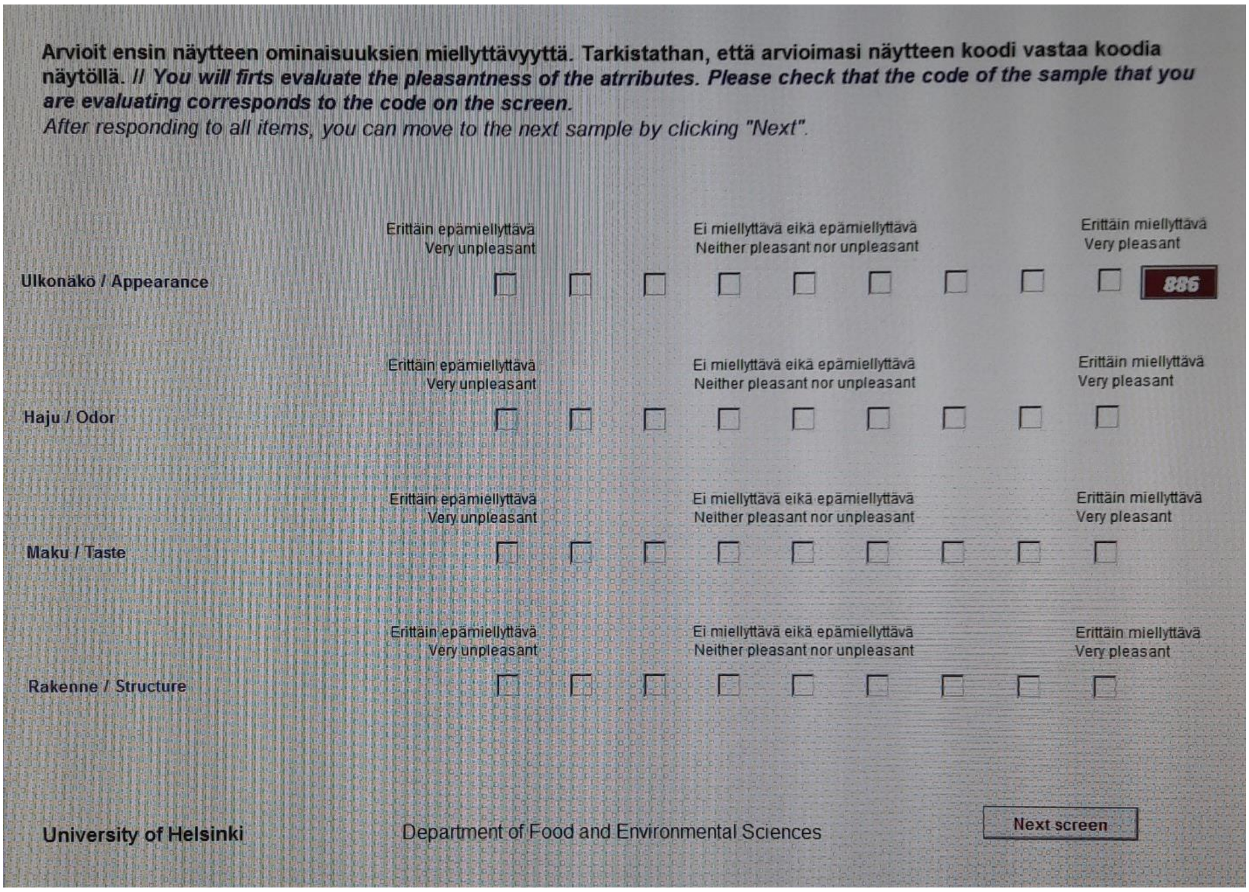
5. Millaista lakritsia syöt yleensä? Rastita kaikki vaihtoehdot, jotka pätevät sinuun. /
What kind of liquorice do you usually eat? Please check all items that apply to you.

- ☐ Peruslakritsia / *Plain liquorice (without special flavors or fillings)*
- ☐ Salmiakkilakritsia / *Salmiac flavored liquorice*
- ☐ Täytelakritsia (esim. ”Sukulaku”) / *Liquorice with filling (e.g. ”Sukulaku”)*
- ☐ Liitulakua / *Liitulaku*
- ☐ Kuorrutettua lakritisia (esim. suklaalla) / *Liquorice with coating (with chocolate)*
- ☐ Lakritsinauhaa (esim. ”Metrilaku”) / *Ribbon-like liquorice (e.g. ”Metrilaku”)*
- ☐ Englanninlakritsia / *Liquorice allsorts*
- ☐ Muunlaista lakritsia. Kirjoita alle. / *Other kind of liquorice. Please describe below.*

  ____

Täytettyäsi lomakkeen siirry arvioimaan lakritsinäytteitä tietokoneella. Kun ohjelma kysyy osallistujanumeroasi, **anna alla oleva numero.** / *When you have completed the questionnaire, start evaluating the liquorice samples with the computer. When the program asks your panelist code,* ***please select the code below.*** **Arvioijanumerosi / Your panelist code: _________________________**

Appendix 6. Consumer test questions



Arvioit ensin näytteen ominaisuuksien miellyttävyyttä. Tarkistathan, että arvioimasi näytteen koodi vastaa koodia näytöllä. // *You will firts evaluate the pleasantness of the atrributes. Please check that the code of the sample that you are evaluating corresponds to the code on the screen.*
After responding to all items, you can move to the next sample by clicking "Next".

	Erittäin epämiellyttävä Very unpleasant				Ei miellyttävä eikä epämiellyttävä Neither pleasant nor unpleasant				Erittäin miellyttävä Very pleasant	
Ulkonäkö / Appearance	☐	☐	☐	☐	☐	☐	☐	☐	☐	886
Haju / Odor	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Maku / Taste	☐	☐	☐	☐	☐	☐	☐	☐	☐	
Rakenne / Structure	☐	☐	☐	☐	☐	☐	☐	☐	☐	

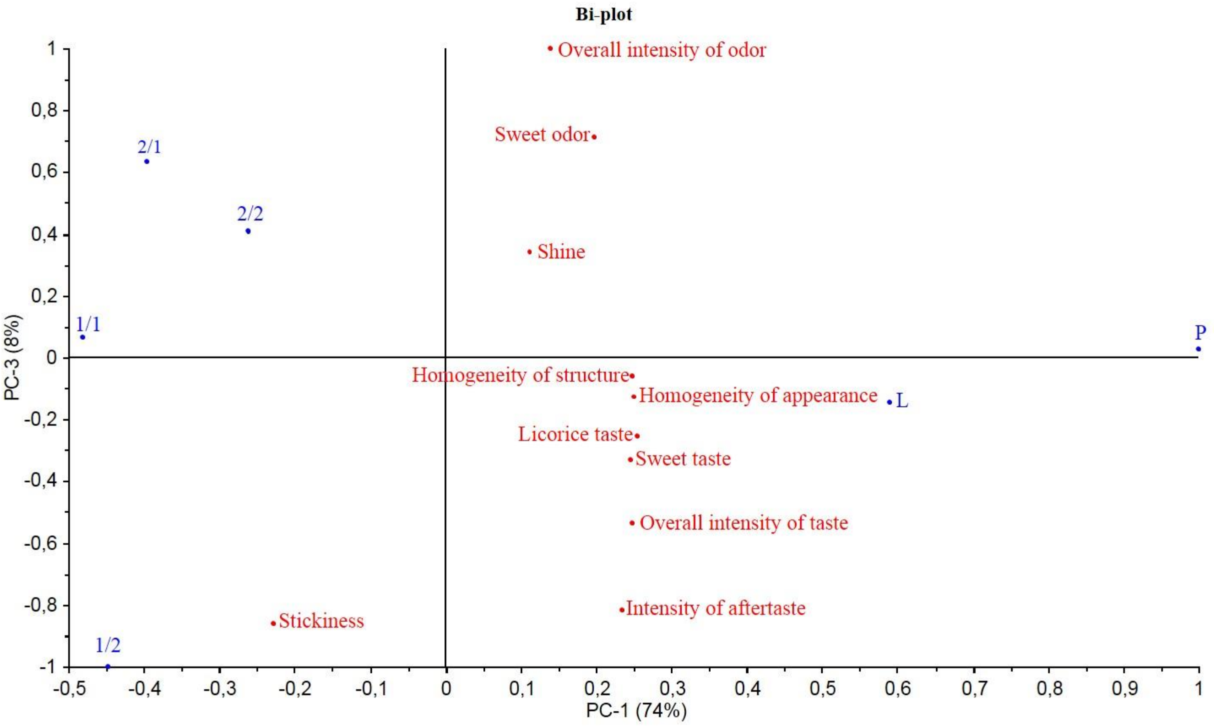
University of Helsinki Department of Food and Environmental Sciences Next screen

Appendix 7. One-way ANOVA and multiple comparison for GDA

		Homogeneity of appearance	Shine	Overall intensity of odor	Sweet odor	Homogeneity of structure	Stickiness	Overall intensity of taste	Sweetness	Licorice taste	Intensity of aftertaste
1/1	1/2	0,993	0,998	0,974	1,000	0,881	0,943	0,494	1,000	0,664	0,107
	2/1	0,998	0,952	0,996	0,999	0,998	0,992	1,000	0,982	0,993	0,999
	2/2	0,370	0,312	0,999	0,826	0,966	1,000	0,954	1,000	0,826	0,954
	P	0,000	0,000	0,999	0,085	0,000	0,291	0,000	0,000	0,000	0,000
	L	0,000	0,084	0,660	0,996	0,000	0,850	0,000	0,000	0,000	0,000
1/2	1/1	0,993	0,998	0,974	1,000	0,881	0,943	0,494	1,000	0,664	0,107
	2/1	1,000	0,998	0,808	0,983	0,631	0,670	0,330	0,927	0,935	0,038
	2/2	0,730	0,579	0,886	0,648	1,000	0,902	0,948	1,000	1,000	0,526
	P	0,000	0,000	0,886	0,038	0,000	0,034	0,000	0,000	0,000	0,005
	L	0,000	0,026	0,213	0,967	0,000	0,299	0,042	0,000	0,000	0,480
2/1	1/1	0,998	0,952	0,996	0,999	0,998	0,992	1,000	0,982	0,993	0,999
	1/2	1,000	0,998	0,808	0,983	0,631	0,670	0,330	0,927	0,935	0,038
	2/2	0,641	0,843	1,000	0,960	0,807	0,998	0,866	0,959	0,986	0,804
	P	0,000	0,000	1,000	0,203	0,000	0,650	0,000	0,000	0,000	0,000
	L	0,000	0,006	0,915	1,000	0,000	0,991	0,000	0,000	0,000	0,000
2/2	1/1	0,370	0,312	0,999	0,826	0,966	1,000	0,954	1,000	0,826	0,954
	1/2	0,730	0,579	0,886	0,648	1,000	0,902	0,948	1,000	1,000	0,526
	2/1	0,641	0,843	1,000	0,960	0,807	0,998	0,866	0,959	0,986	0,804
	P	0,000	0,000	1,000	0,696	0,000	0,363	0,000	0,000	0,000	0,000
	L	0,000	0,000	0,847	0,979	0,000	0,904	0,002	0,000	0,000	0,008
P	1/1	0,000	0,000	0,999	0,085	0,000	0,291	0,000	0,000	0,000	0,000
	1/2	0,000	0,000	0,886	0,038	0,000	0,034	0,000	0,000	0,000	0,005
	2/1	0,000	0,000	1,000	0,203	0,000	0,650	0,000	0,000	0,000	0,000
	2/2	0,000	0,000	1,000	0,696	0,000	0,363	0,000	0,000	0,000	0,000
	L	1,000	0,000	0,847	0,253	1,000	0,938	0,337	1,000	0,067	0,432
L	1/1	0,000	0,084	0,660	0,996	0,000	0,850	0,000	0,000	0,000	0,000
	1/2	0,000	0,026	0,213	0,967	0,000	0,299	0,042	0,000	0,000	0,480
	2/1	0,000	0,006	0,915	1,000	0,000	0,991	0,000	0,000	0,000	0,000
	2/2	0,000	0,000	0,847	0,979	0,000	0,904	0,002	0,000	0,000	0,008
	P	1,000	0,000	0,847	0,253	1,000	0,938	0,337	1,000	0,067	0,432

Attribute	Sig.
Homogeneity of appearance	0,000
Shine	0,000
Overall intensity of odor	0,371
Sweet odor	0,045
Homogeneity of structure	0,000
Stickiness	0,060
Overall intensity of taste	0,000
Sweetness	0,000
Licorice taste	0,000
Intensity of aftertaste	0,000

Appendix 8. Principal component analysis for GDA (components 1 & 3)



Appendix 9. One-way ANOVA and multiple comparison for consumer test 1

Appearance		Sig	Odor		Sig	Taste		Sig	Structure		Sig	Overall		Sig
1/1	1/2	1,000	1/1	1/2	1,000	1/1	1/2	0,916	1/1	1/2	0,758	1/1	1/2	0,578
	2/1	0,799		2/1	0,976		2/1	1,000		2/1	0,997		2/1	0,998
	2/2	1,000		2/2	0,982		2/2	0,849		2/2	1,000		2/2	0,994
	P	0,000		P	0,021		P	0,000		P	0,000		P	0,000
	L	0,000		L	0,699		L	0,000		L	0,000		L	0,000
1/2	1/1	1,000	1/2	1/1	1,000	1/2	1/1	0,916	1/2	1/1	0,758	1/2	1/1	0,578
	2/1	0,921		2/1	0,900		2/1	0,941		2/1	0,953		2/1	0,847
	2/2	1,000		2/2	0,915		2/2	0,249		2/2	0,886		2/2	0,886
	P	0,000		P	0,008		P	0,000		P	0,000		P	0,000
	L	0,000		L	0,498		L	0,000		L	0,000		L	0,000
2/1	1/1	0,799	2/1	1/1	0,976	2/1	1/1	1,000	2/1	1/1	0,997	2/1	1/1	0,998
	1/2	0,921		1/2	0,900		1/2	0,941		1/2	0,953		1/2	0,847
	2/2	0,799		2/2	1,000		2/2	0,808		2/2	1,000		2/2	1,000
	P	0,000		P	0,155		P	0,000		P	0,000		P	0,000
	L	0,000		L	0,982		L	0,000		L	0,000		L	0,000
2/2	1/1	1,000	2/2	1/1	0,989	2/2	1/1	0,849	2/2	1/1	1,000	2/2	1/1	0,994
	1/2	1,000		1/2	0,915		1/2	0,249		1/2	0,886		1/2	0,886
	2/1	0,799		2/1	1,000		2/1	0,808		2/1	1,000		2/1	1,000
	P	0,000		P	0,141		P	0,000		P	0,000		P	0,000
	L	0,000		L	0,976		L	0,000		L	0,000		L	0,000
P	1/1	0,000	P	1/1	0,021	P	1/1	0,000	P	1/1	0,000	P	1/1	0,000
	1/2	0,000		1/2	0,008		1/2	0,000		1/2	0,000		1/2	0,000
	2/1	0,000		2/1	0,155		2/1	0,000		2/1	0,000		2/1	0,000
	2/2	0,000		2/2	0,141		2/2	0,000		2/2	0,000		2/2	0,000
	L	0,036		L	0,527		L	0,849		L	0,995		L	0,965
L	1/1	0,000	L	1/1	0,699	L	1/1	0,000	L	1/1	0,000	L	1/1	0,000
	1/2	0,000		1/2	0,498		1/2	0,000		1/2	0,000		1/2	0,000
	2/1	0,000		2/1	0,982		2/1	0,000		2/1	0,000		2/1	0,000
	2/2	0,000		2/2	0,976		2/2	0,000		2/2	0,000		2/2	0,000
	P	0,036		P	0,527		P	0,849		P	0,995		P	0,965

Pleasantness	Significance between groups
Appearance	0,000
Odor	0,010
Taste	0,000
Structure	0,000
Overall	0,000

Appendix 10. One-way ANOVA and multiple comparison for consumer test 2

Appearance		Sig	Odor		Sig	Taste		Sig	Structure		Sig	Overall		Sig
3/1	3/2	1,000	3/1	3/2	0,975	3/1	3/2	0,989	3/1	3/2	0,930	3/1	3/2	0,935
	4/1	0,060		4/1	0,688		4/1	0,534		4/1	0,391		4/1	0,824
	4/2	0,297		4/2	0,958		4/2	0,040		4/2	0,061		4/2	0,114
	P	0,000		P	0,032		P	0,000		P	0,000		P	0,000
	L	0,000		L	0,773		L	0,000		L	0,000		L	0,000
3/2	3/1	1,000	3/2	3/1	0,975	3/2	3/1	0,989	3/2	3/1	0,930	3/2	3/1	0,935
	4/1	0,031		4/1	0,981		4/1	0,188		4/1	0,048		4/1	0,255
	4/2	0,188		4/2	1,000		4/2	0,005		4/2	0,003		4/2	0,007
	P	0,000		P	0,211		P	0,000		P	0,000		P	0,000
	L	0,000		L	0,301		L	0,000		L	0,000		L	0,000
4/1	3/1	0,060	4/1	3/1	0,688	4/1	3/1	0,534	4/1	3/1	0,391	4/1	3/1	0,824
	3/2	0,031		3/2	0,981		3/2	0,188		3/2	0,048		3/2	0,255
	4/2	0,981		4/2	0,999		4/2	0,811		4/2	0,954		4/2	0,774
	P	0,000		P	0,627		P	0,000		P	0,000		P	0,000
	L	0,000		L	0,062		L	0,000		L	0,000		L	0,000
4/2	3/1	0,297	4/2	3/1	0,958	4/2	3/1	0,040	4/2	3/1	0,061	4/2	3/1	0,114
	3/2	0,188		3/2	1,000		3/2	0,005		3/2	0,003		3/2	0,007
	4/1	0,981		4/1	0,990		4/1	0,811		4/1	0,954		4/1	0,774
	P	0,000		P	0,253		P	0,000		P	0,000		P	0,000
	L	0,000		L	0,253		L	0,000		L	0,000		L	0,000
P	3/1	0,000	P	3/1	0,032	P	3/1	0,000	P	3/1	0,000	P	3/1	0,000
	3/2	0,000		3/2	0,211		3/2	0,000		3/2	0,000		3/2	0,000
	4/1	0,000		4/1	0,627		4/1	0,000		4/1	0,000		4/1	0,000
	4/2	0,000		4/2	0,253		4/2	0,000		4/2	0,000		4/2	0,000
	L	0,009		L	0,000		L	0,999		L	1,000		L	0,967
L	3/1	0,000	L	3/1	0,773	L	3/1	0,000	L	3/1	0,000	L	3/1	0,000
	3/2	0,000		3/2	0,301		3/2	0,000		3/2	0,000		3/2	0,000
	4/1	0,000		4/1	0,062		4/1	0,000		4/1	0,000		4/1	0,000
	4/2	0,000		4/2	0,253		4/2	0,000		4/2	0,000		4/2	0,000
	P	0,009		P	0,000		P	0,999		P	1,000		P	0,967

Pleasantness	Significance between groups
Appearance	0,000
Odor	0,001
Taste	0,000
Structure	0,000
Overall	0,000

Appendix 11. Results from the instrumental testing

Water activity

Sample	Water activity (AquaLab)	Water activity (Novasina)	Average water activity	Average standard deviation
1/1	0,693	0,670	0,681	0,022
1/2	0,684	0,665	0,675	0,026
2/1	0,681	0,664	0,672	0,022
2/2	0,684	0,658	0,671	0,022
P	0,638	0,615	0,626	0,024
L	0,561	0,552	0,557	0,019

Extension

Sample	Force at maximum load (N)	Displacement at maximum load (mm)	Tensile strength (N/mm)
1/1	2,87	13,44	0,21
1/2	1,93	10,20	0,19
2/1	2,55	11,82	0,22
2/2	1,94	9,51	0,20

Compression

Sample	Maximum slope	Compressive displacement (mm)	Primary force measurement (N)	Compressive strength (N/mm)
1/1	65,38	2,00	14,95	7,48
1/2	56,39	2,45	12,34	5,04
2/1	70,76	2,39	10,08	4,22
2/2	66,42	2,61	9,76	3,74
P	38,51	2,62	32,90	12,55
L	58,97	3,92	21,17	5,40